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ABSTRACTS

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Oral Abstracts

Role of Granulocyte Transfusions in Hematopoetic Stem Cell Transplantation: Experience from a Tertiary Care Hospital in North India

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Introduction: Granulocyte transfusions is an area of controversy in heamatopoietic stem cell transplantation.Bacterial and fungal infections still remain an important cause of mortality in patients with hematological malignancies and in recipients of hematopoietic stem cell transplants (HSCT) especially in developing countries like India. Granulocyte transfusions (GTX) from healthy donors may lead to early clearance of index infection and thus prevent mortality. The aim of the present study was to evaluate the efficacy of GTX in combating life-threatening infections and preventing mortality in patients of hematological disorders/recipients of HSCT with severe neutropenia.
Aims & Objectives: To determine the role of Granulocyte transfusions in severe neurogenic condition with associated MDROs/Fungal infection with severe sepsis/ septic shock while patients undergoing Hematopoetic stem cell transplantation.
Materials & Methods: This study was a prospective, observational analysis of patients with different hematological disorders undergoing autologous or allogenic HSCT, who received GTX from January 2017- July 2022. All patients had an Absolute neutrophil Count (ANC) $< 0.5 \times 10^9$/L and a life threatening sepsis defined by presence of hemodynamic instability/impending septic shock/continuous high fever despite the use of the highest line of antimicrobials.
Result: A total of 57 granulocyte collections were done for 50 infectious episodes (IEs) in 50 patients undergoing autologous 22/50 (44%) or Allogenic 28/50 (56%), including 5 haplo-identical HSCT. R/R Hodgkin's lymphoma (8/22) & Multiple myeloma (6/22) were the most common indications for Autologous HSCT & AML(14/28) & SAA (10 / 28) were most common indications for allogenic HSCT. Multi- drug resistant organisms (MDROs) were observed in 24/50 IEs (48%) and fungal infections were seen in 5/50 IEs (10%) Resolution of index infection after GTX was seen in 46/50 IEs (92%) and the 30 day overall survival (OS) was 90%. OS was significantly higher in patients who received GTX within 7 days of neutropenic sepsis ($p = 0.01$). Patients with MDROs who received early GTX therapy had a better OS as compared to those who received late GTX ($p = 0.02$). GTX were well tolerated and only 3 patients' developed mild features of transfusion related acute lung injury (TRALI) which was managed conservatively, and 1 patient demonstrated hypocalcemic tetany.
Conclusions: GTX may be of particular relevance in countries like India, where the incidence of infections is very high in neutropenic patients and there is an increasing emergence of MDROs.

Quinolone Prophylaxis and Carbapenem-Resistant Infection in Patients Undergoing Hematopoietic Stem Cell Transplant

Rahul Naithani, Pronamee Borah, Sangeeta Pathak, Nitin Dayal, Bansidhar Tarai

Introduction: Infections remain a major cause of morbidity and mortality in patients undergoing hematopoietic stem cell transplant.

Aims & Objectives: Primary outcome measure was infection related mortality. Secondary outcome measures included documented infections, blood stream infections (BSI), incidence of gram positive and gram-negative sepsis and duration of hospital stay.
Materials & Methods: Retrospective observational study. Total 219 [124 patients with autologous stem cell transplant (ASCT) and 95 patients with allogeneic stem cell transplant (AlloSCT)] were included. The first 100 patients undergoing transplant received antibiotic prophylaxis and next 119 patients did not receive antibiotic prophylaxis.
Result: Baseline characteristics were compared between the two groups. Both groups had comparable duration of fever and hospitalization duration. Documented infection was significantly lower in patients who received antibiotic prophylaxis (29% vs 42.9%; p = 0.034). The patients who did not receive antibiotic prophylaxis had higher rates of gram negative (34.5% vs 22%; p = 0.043) and carbapenem resistant enterobacteriacae sepsis (21% vs 1%; p = 0.001) but there was no difference in the rates of gram-positive sepsis or bacteremia.
Number of febrile episodes, duration of fever, documented infections, BSI and gram-positive BSI were similar in both groups. Gram-negative infection and carbapenem resistance were significantly lower in prophylaxis group in ASCT patients. However, carbapenem resistance was significantly lower in prophylaxis group (2% vs 23.9%) in Allo SCT group. Antibiotic prophylaxis was not associated with reduction in mortality (p = 0.258).
Conclusions: Antibiotic prophylaxis significantly reduces documented bacterial infections and incidence of carbapenem resistance but did not reduce mortality.

Role of Microrna in Hydroxyurea Mediated HbF Induction in Sickle Cell Anemia Patients

Neha Kargutkar, Madhavi Sawant-Mulay, Priya Hariharan, Chandrakala S, Anita Nadkarni

Introduction: Sickle cell anemia (SCA) is the commonest monogenic disorder in India. Hydroxyurea (HU) is the drug for SCA however the mechanism through which it induces HbF is unclear. Role of miRNAs is well characterised in regulating HBG2 globin gene expression.
Aims & Objectives: The study aimed to investigate the role of miRNAs in HU mediated HbF induction in Sickle cell anemia patients.

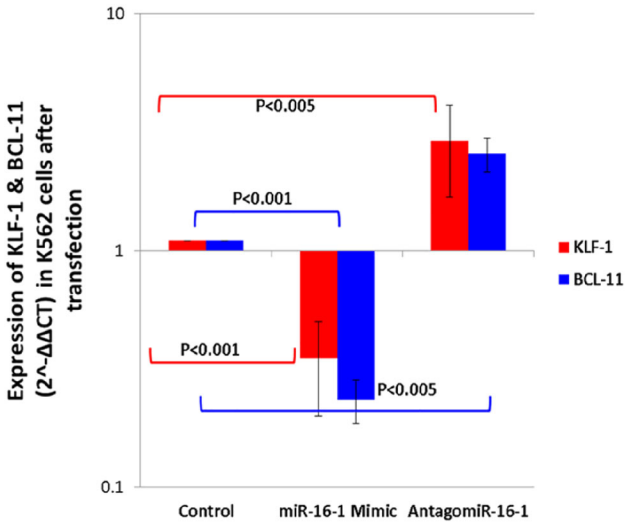

Fig. 1 Expression of KLF-1 and BCL11A after transfection of miR-16–1 mimic and antagomir in K562 cells

Materials & Methods: Study enrolled 30 normal controls and 30 SCA patients at baseline, 20 patients after 3 and 6 months of hydroxyurea (HU) therapy. HbF levels were studied on HPLC. Expression of 10 miRNAs were identified in SCA patients. Target genes of miRNAs were predicted and functionally validated in K562 cell model and CD34+ cells of SCA patients. miRNA-mRNA network and protein–protein interaction (PPI) was constructed to understand biological processes and pathways.
Result: The mean HbF levels and HBG2 globin gene levels significantly increased after 3 and 6 months of HU therapy in SCA patients compared to baseline ($p < 0.0001$). 8 miRNAs were significantly up-regulated while 2 were down-regulated. The increase in miR-210, miR-16–1, and miR-29a expression and decrease in miR-96 expression were strongly associated with the HU mediated HbF induction. Post HU therapy, decreased miR-96 expression was observed; which might be due to interference of miR-96 binding to HBG2-globin mRNA by HU, facilitating HbF expression. The miR-210 expression was enhanced in association with erythroid differentiation and induction to HbF production. In silico tools predicted BCL11A and KLF as a target gene of miRNAs. We transfected mimic and antimiRs of miR-16–1 and miR-96 in K562 cells and SCA patient derived CD34 + cells and found that these miRNAs induce HBG2-globin gene expression by modulating BCL11A, KLF1 (Fig. 1). We constructed PPI and miRNA network model and found that target genes are significantly enriched in erythropoiesis and cell cycle regulation pathways.
Conclusions: The study suggests the role of miR-210, miR16-1, miR-29a, and miR-96 in HBG2-globin gene regulation leading to HbF induction. Identification of the relevant protein targets might be useful for understanding the HU mediated HbF induction.

Experience of ITI Therapy for Hemophilia Patients from a Tertiary Care Center of Eastern India: A One Year Follow Up

Prerna Pramanik, Maitreyee Bhattacharyya

Introduction: The development of inhibitor is the main complication of hemophilia treatment in patients receiving factor replacement as management. The mainstay of therapy in patients who develop inhibitor is to eliminate the inhibitor entirely. This can be achieved by immune tolerance induction therapy which comprises regular F8/F9 infusions in high dose. There are only a few studies on ITI from India and hardly any from Eastern India.
Aims & Objectives: To evaluate the outcome and clinical profile of hemophilia patients receiving ITI therapy and their follow up.
Materials & Methods: It is a retrospective analysis of data. The study describes the clinical profile and outcome of eleven severe hemophilia patients who had developed inhibitor after few years of factor replacement therapy and thereafter started on ITI therapy. They were followed and serial monitoring of their inhibitor levels were done.
Result: All of the eleven patients were severe haemophilia patients. Six of them initially were on prophylactic factor replacement therapy, five on demand therapy. Four out of eleven suffered at least one episode of breakthrough bleed during which majority were administered FEIBA, one received novoseven. Dose increment was required in 40% of the cases. Majority patients' inhibitor level increased initially after starting ITI therapy. Six showed complete response and are off ITI therapy now. One patient was partial success, one was failure and now receiving injection Rituximab. Age at ITI start varied from 1 year 5 months to 20 years, baseline inhibitor level varied from 6 BU/ml to 1485 BU/ml and dose of ITI varied from 50U/kg to 100U/kg. No correlation between age at ITI start, baseline inhibitor level, dose of ITI and outcome was found.
Conclusions: ITI therapy was successful in around 60% of cases and should be tried in all hemophilia patients with inhibitor.

Concizumab Prophylaxis in Patients with Haemophilia A or B with Inhibitors: Efficacy and Safety Results from the Primary Analysis of the Phase 3 EXPLORER7 Trial

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Introduction: Concizumab is a subcutaneously administered anti-tissue factor pathway inhibitor (TFPI) antibody in development as once-daily prophylaxis for all haemophilia patients. Explorer7 (NCT04083781) primary analysis results are presented.
Aims & Objectives: Explorer7 assessed Concizumab efficacy and safety in haemophilia A/B with inhibitor (HAwI/HBwI) patients.
Materials & Methods: Patients were randomised 1:2 to no prophylaxis (arm 1; $\geq$ 24 weeks) or concizumab prophylaxis (arm 2; $\geq$ 32 weeks), or assigned to concizumab prophylaxis (arms 3&4). After treatment restart following pause due to thromboembolic events, patients received a 1.0 mg/kg concizumab loading dose, followed by an initial 0.20 mg/kg daily dose, with potential adjustment to 0.15 or 0.25 mg/kg based on plasma concizumab concentration at week 4. The primary analysis compared number of treated spontaneous and traumatic bleeding episodes between arms 1 and 2 (using negative binomial regression). Safety, patient-reported outcomes, and pharmacokinetics/pharmacodynamics were assessed. Informed consent/ethics committee approval were obtained.
Result: Of 133 enrolled patients, 33 were randomised to concizumab (arm 2) and 19 to no prophylaxis (arm 1) (28 and 14 completed $\geq$ 32/24 weeks of treatment at the primary analysis cut-off, respectively); the remaining 81 were assigned to concizumab (arms 3&4). Estimated mean annualised bleeding rate (ABR) was 1.7 (95% CI, 1.0–2.9) for concizumab versus 11.8 (95% CI, 7.0–19.9) for no prophylaxis (ABR ratio, 0.14 [95% CI, 0.07–0.29]; $P < 0.001$). Median ABR on concizumab was 0 (Fig. 1). Twenty one (63.6%) concizumab patients had zero treated bleeds at 24 weeks (including those who discontinued before 24 weeks) versus two (10.5%) on no prophylaxis. No thromboembolic events were reported after treatment restart (Table 1). Positive trends were observed across 36-Item Short-Form Health Survey (SF-36v2) domains with concizumab. Concizumab exposure was stable over time.
Conclusions: Concizumab prophylaxis effectively reduced ABR versus no prophylaxis and was considered safe and well tolerated in HAwI/HBwI patients.

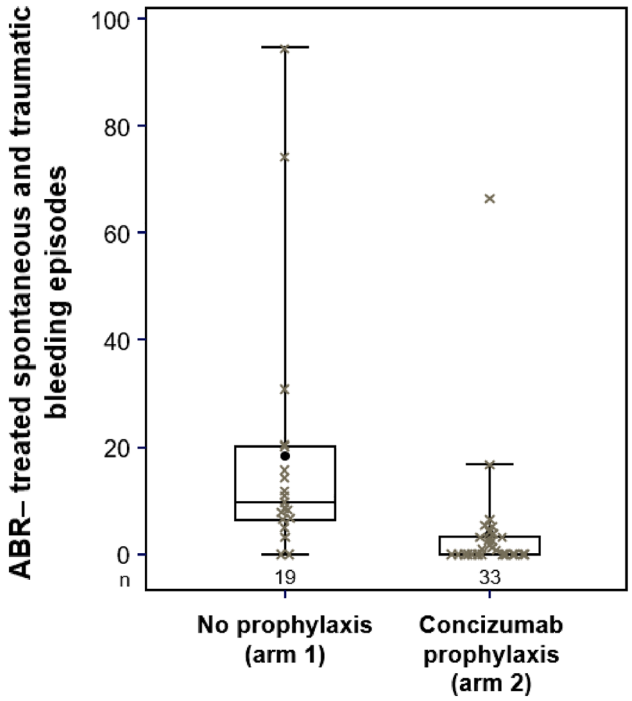


Fig. 1 Annualised bleeding rate for no prophylaxis *versus* concizumab prophylaxis at the primary analysis cut-off* of the phase 3 explorer7 trial in patients with haemophilia A/B with inhibitors—descriptive results for primary endpoint. *Primary analysis cut-off is defined as when all patients in arm 1 have completed visit 9a (week 24) or withdrawn, and all patients in arm 2 have completed visit 10a (week 32) or withdrawn. The primary analysis included: all 'no prophylaxis' data (i.e., before, during and after the treatment pause); all concizumab data after the pause up until the primary analysis cut-off (or treatment discontinuation), as well as concizumab data from before the pause for arm 2 patients that did not restart. Periods with use of ancillary therapy were excluded. The filled circle: mean; the top/bottom of the box: 1st/3rd quartile; the line inside the box: median; the whiskers: 5th and 95th percentiles; the 'x's represent the individual values. ABR, annualised bleeding rate

Authorship Diversity in Haematology-Related Cochrane Systematic Reviews: Inequities in Global Representation

Jyotirmoy Biswas, Arkadeep Dhali, Roger Rathna, Christopher DSouza

Introduction: The need for promoting diversity and equitable authorship representation in academics faces increasing recognition, with some articles pointing out the lack of diversity in specific fields. Currently, there are no such articles scrutinizing the author diversity in the field of Haematology. Cochrane systematic reviews are perceived worldwide to be amongst the highest quality of evidence available, thereby its conclusions often impact policy and practice globally. However, little is known about the current state of authorship diversity in Haematology-related Cochrane reviews.
Aims & Objectives: This study sought to determine the gender and country diversity in authorship representation in the authorship of Cochrane systematic reviews related to Haematology.
Materials & Methods: We searched and extracted data from the Cochrane Library on 20 May 2022 using 'topic: Haematology', and included published reviews, protocols, and withdrawn publications. We extracted authors' details and searched online to determine their gender, attempting to capture at least one webpage demonstrating it. Authors whose gender could not be ascertained were excluded from gender-based analyses. For graphical representation, we used a chloropleth-style map.
Result: One hundred and thirty nine publications with a total of 937 authors were included in the current study. The leading five represented nations (Figure 1) in authorship were Germany (n = 396, 42.3%), United Kingdom (n = 167, 18%), China (n = 65, 7%), United States of America (n = 57, 6.2%), and Israel (n = 48, 5.2%). First authors were mostly represented by Germany (n = 58, 41.7%), followed by United Kingdom (n = 22, 15.8%), China (n = 14, 10%), United States of America (n = 8, 5.7%), and Israel (n = 8, 5.7%). Only twenty-one (2.24%) authors from low and low-middle-income countries had authorship representation.
Male (n = 438) to female (n = 499) ratio in this study was 1:13. There were 63 (45.3%) male and 76 (54.7%) female first authors. Women (n = 80) constituted 57.5% of all the corresponding authors. Fifty-two

Table 1 Adverse events reported prior to the primary analysis cut-off* of the concizumab phase 3 explorer7 trial in patients with haemophilia A/B with inhibitors

	No prophylaxis (arm 1)		Concizumab prophylaxis (arm 2)		Concizumab prophylaxis (all patients†)	
	n (%)	E [R]	n (%)	E [R]	n (%)	E [R]
Number of patients	19		33		127	
Patient years of exposure	12		32		112	
Total events	8 (42.1)	25 [2.1]	20 (60.6)	60 [1.9]	80 (63.0)	356 [3.2]
Serious events	3 (15.8)	5 [0.4]	6 (18.2)	9 [0.3]	14 (11.0)	18 [0.2]
Fatal events‡	1 (5.3)	1 [0.1]	2 (6.1)	4 [0.1]	2 (1.6)	4 [0.0]
Drug withdrawn§	0		2 (6.1)	2 [0.1]	4 (3.1)	4 [0.0]
Thromboembolic events	0		1 (3.0)	1 [0.0]	1 (0.8)	1 [0.0]
Thromboembolic events after treatment restart¶	0		0		0	
Hypersensitivity-type reaction	0		1 (3.0)	1 [0.0]	2 (1.6)	2 [0.0]
Injection site reaction	0		6 (18.2)	9 [0.3]	26 (20.5)	48 [0.4]

*For arm 1, this includes data from randomisation until the start of concizumab treatment. For concizumab, this includes data from when the patient started concizumab treatment until 7 weeks after the treatment pause, in addition to data from when concizumab treatment was restarted until the primary analysis cut-off.

†Includes arm 1 patients who started concizumab treatment in the extension part of the trial, arm 2 patients, and the patients who received non-randomised concizumab treatment.

‡The adverse events with a fatal outcome were pneumonitis in a patient on no prophylaxis, and COVID-19 and a road traffic accident in the two patients receiving concizumab prophylaxis (both considered unlikely related to concizumab treatment). There were also two adverse events with a fatal outcome during the treatment pause: haematoma (with co-reported vena cava thrombosis, retinal vascular occlusion, and urinary tract obstruction) and gastrointestinal haemorrhage (not included in Table 1).

§The adverse events leading to drug withdrawal were congestive cardiomyopathy, a non-fatal renal infarct (which was one of the thromboembolic events that led to the treatment pause and subsequent protocol amendment), hypersensitivity, and COVID-19.

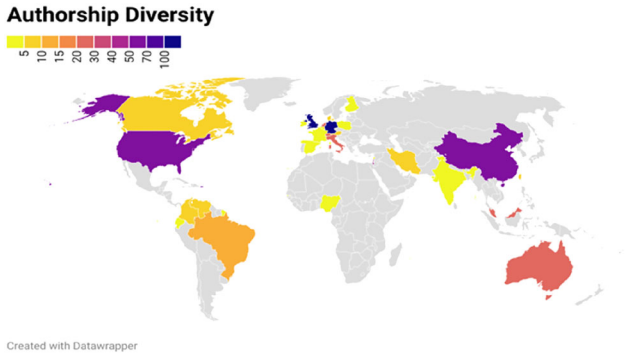
¶Includes data from after the treatment restart only, i.e., data from prior to the protocol amendment are excluded (arm 2: n = 29, PYE = 24; all patients on concizumab: n = 112, PYE = 90).

E, number of events; R, rate of events; n, number of patients; PYE, patient years of exposure.

(37.4%) studies didn't have female representation in any lead author (corresponding or first author) positions. Twelve (8.6%) studies didn't have any female authors at all.

Conclusions: Authors from high-income countries continue to be the largest contributors to Cochrane systematic reviews in Haematology. There is an extremely poor representation of authors from low and low-middle-income countries. Despite the fact that only less than 40%

of practicing hematologists are female, an overwhelming representation of women was noted in the overall as well as lead authorship.



Automation vs. Manual Platelet Count: An Audit of a Real-Life Scenario in a Tertiary Care Center in India

Shruti Mishra, AshisGupta, Kailash Kumar

Introduction: Platelets are small anucleated granular cytoplasmic fragments of megakaryocytes. While thrombocytosis begets thrombotic events, thrombocytopenia leads to bleeding disorders. Hence, an accurate platelet count is necessary for planning transfusions in cases of thrombocytopenia. The immunological-based gold standard platelet estimation is not routinely feasible, and automation remains a faster alternative.

Aims & Objectives: The main aim of this study was to audit the platelet count and platelet indices generated by five-part analyzer and compare the correlation with the manual count done on Leishman stained slides.

Materials & Methods: This prospective cross-sectional study was done in the Central Laboratory of Sir Sunderlal Hospital, BHU. 1795 samples were collected, in whom slides were reviewed, from a total of 19,028 samples processed for CBC. The platelet count on the machine and manual counts were recorded. The analyzer works on the principle of impedance. The manual count was done on thin Leishman-stained smears. All the parameters of platelets were noted, like count, mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW).

All the data collected were coded and entered in Microsoft Excel sheet which was re-checked and analysed using SPSS v 22. Spearman correlation was done to find out correlation between two quantitative variables. A p value of <0>

Result: 927 (51.6%) patients belonged to the young adult age group (15–47 years). 60.1% were males, and the rest were female patients. 82.2

Conclusions: Patients with marked thrombocytopenia and high MPV (> 12 fl) on machine should be undergo slide review. Transfusion-related decisions can then be made based on the manual count, thus avoiding unnecessary transfusions and exposure (Table 1).

Table 1 Correlation of manual and machine platelet counts in different scenarios

Platelet count	Machine platelet count	
	Correlation coefficient	P-value
Thrombocytopenia		
Manual platelet count	0.859	< 0.001*
Normal platelet count		
Manual platelet count	0.681	< 0.001*
Thrombocytosis		
Manual platelet count	0.997	< 0.001*
> 12 fl		
Manual platelet count	0.495	< 0.001*
≤ 12 fl		
Manual platelet count	0.883	< 0.001*
Platelet count of 10,000–20,000		
Manual platelet count	0.485	< 0.001*
Platelet count of < 10,000		
Manual platelet count	0.282	0.087

Exploring the Alterations in Erythrocytes and Haemoglobin in Women with PCOS: A Follow Up Study

Ipsita Chakraborty, Sutithi Dey, Ayantika Paul, Pratip Chakraborty, Rajen Haldar

Introduction: The etiology of polycystic ovary syndrome (PCOS) is still unknown and search for causative factor(s) is proving elusive, and it is agreed that hyperandrogenism is the base of the syndrome. Testosterone is a hematopoietic hormone with dose-dependent stimulatory effect on erythropoiesis and oxidative stress is essentially correlated with features like hyperinsulinemia, hypertension, and dyslipidemia in PCOS women.

Aims & Objectives: The aim of this study was to explore the effects of polycystic ovary pathology on hemoglobin and erythrocytes before and after treatment.

Materials & Methods: Twenty (age 22–35 years; BMI 28–32 kg/sq m) women diagnosed with PCOS as per Rotterdam Criteria, 2003 were volunteered in this study; thirteen of them (clomiphene-metformin group) received clomiphene citrate (CC) 50 mg daily from day 3–7, and metformin 500 mg twice daily for three ovulatory cycles, and 7 women received CC alone for similar duration and dosage. Ten Age-matched women undergoing IVF due to male factor infertility diagnosed as per AUA/ASRM guidelines was treated as control. Anthropometric measurements and biochemical parameters were evaluated in each patient. Markers of membrane damage including lipid peroxidation, carbonyl formation along with membrane morphological view through SEM were the heart of the study. Co-oxidation of hemoglobin in presence of NBT as well as the spectral analysis of met Hb formation were also compared for specific PCOS correlating with their therapeutic functionality and the healthy control. Osmotic fragility was studied in all samples exposing the differences of hemolysis among them.

Result: Lipid peroxidation and carbonyl formation were increased in PCOS compared to the control, which significantly abated post treatment. SEM exposed the exaggerated morphological deviation in PCOS as compared to treated and control group. Rate of co- oxidation was higher, whereas the osmotic fragility was lower in PCOS, which was almost reversed back to normal after treatment.

Conclusions: The study provided strong evidence of underlying association betweenthe pathophysiology of PCOS and the alterations of physicochemical properties of erythrocytes and hemoglobin which were triggered affirmatively by Treatments. Hereby weconclude that after providing treatments the dysregulations of PCOS were certainly modifying back.

Reticulocyte Haemoglobin Equivalent as a Potential Marker for Diagnosis and Treatment Response of Iron Deficiency Anemia

Manali Satiza, Mahadev Meena, Naresh Midha, Deepak Kumar, Gopal Krishana Bohra, Abhishek HL Purohit

Introduction: Early diagnosis and monitoring of treatment response in iron deficiency anemia(IDA) are challenging. Serum ferritin is a universal standardized parameter used along with conventional markers to diagnose IDA. However, high serum ferritin values are observed in inflammatory states as well as in chronic disease states downplaying its role in screening for IDA as well as for the monitoring treatment response. Reticulocyte Hb equivalent(RET-He) is a reticulocyte parameter that measures the hemoglobin(Hb) content of red blood cells and offers real-time information on iron supply for erythropoiesis.
Aims & Objectives: 1. To study the utility of Ret-He in the diagnosis of IDA and its comparison with conventional iron parameters.
2. To evaluate an early treatment response by Ret-He.
Materials & Methods: This prospective observational study was done on patients attending Haematology clinic at AIIMS, Jodhpur. After obtaining approval from Institute's ethics committee, cases of IDA and healthy age-matched control were enrolled. Complete haemogram and RET-He were performed on an automated Haematology cell counter (Sysmex-XN1000) at the time of diagnosis and after 7–10 days of iron infusion. Comparative analysis was done between RET-He and other parameters to diagnose IDA. A comparative analysis was done between RET-He and Hb to assess response to iron treatment.
Result: A total of 105 adult patients of IDA and 30 controls were enrolled. There was a positive strong correlation between RET-He & Hb and RET-He & serum iron to diagnose IDA ($p < 0.005$). No significant correlation was found between RET-He & ferritin. RET-He was found to be an early indicator to assess response to treatment with a significant and early rise around the 7th day of iron-infusion, whereas a rise in Hb value was observed at around the 15th day of iron-infusion.
Conclusions: Our study highlights that RET-He is an extremely valuable marker for the diagnosis of ID and IDA. This marker also serves as the earliest predictor of response to treatment & hence it is of great clinical utility.

Flow cytometric estimation of platelet derived microparticles isolated by sedimentation and centrifugation protocols in chronic diseases: a comparative study

Shagun Wadhwa, Mrinalini Kotru, Richa Gupta, Priyanka Gogoi, Shiva Narang

Introduction: Platelet derived microparticles (PMPs) have emerged as both, biomarkers and contributors to various chronic diseases. PMPs are 0.1 to 1 micron, small sized fragments which are shed from platelets upon activation or shear stress or due to apoptotic stimuli. Detection of PMPs is cumbersome and there is no simple and standardized technique for their detection yet. Therefore, there is a need to explore simple and sensitive techniques to detect them in blood.
Aims & Objectives: To compare the platelet microparticle (PMP) levels isolated by sedimentation, low speed centrifugation (Platelet Rich Plasma) and high speed centrifugation (Platelet Poor Plasma) in patients with chronic diseases.
Materials & Methods: A comparative cross-sectional study was conducted enrolling 30 patients with chronic diseases like Diabetes, Hypertension, Rheumatoid Arthritis, Systemic Lupus Erythematosus and Chronic Hepatitis. Complete hematological profile and immunophenotyping by flow cytometry was done for detection of PMPs. Antibodies against CD45, CD41, CD61, Annexin V and CD42b were used to distinguish PMPs from platelets. Standardization of microparticle gate was established using 300 nm size calibration beads. The particles that were positive for both—Annexin V and CD42b were taken as PMPs.
Result: PMP levels ranged from 0.11 to 11.48% with a mean ± SD of 3.34 ± 3.20 and a median (IQR) of 1.87 (4.11) in Sedimentation, 0.32–24.03% with a mean ± SD of 4.56 ± 5.00 and a median (IQR) of 3.00 (4.58) in Platelet Rich Plasma (PRP) and 0.62–35.79% with a mean ± SD of 8.95 ± 8.67 and a median (IQR) of 6.44 (5.41) in Platelet Poor Plasma (PPP). The yield of PMPs was highest in PPP in terms of mean, median and range. Also, there was a strong correlation between Annexin V + CD42b (PMP%) (Platelet Rich Plasma) and Annexin V + CD42b (PMP%) (Platelet Poor Plasma), and this correlation was statistically significant (Interclass Correlation Coefficient = 0.66, p = < 0.001).
Conclusions: Centrifugation protocols are superior in isolating platelet microparticles as compared to sedimentation. Low speed centrifugation (Platelet Rich Plasma) and high speed centrifugation (Platelet Poor Plasma) have similar efficacy in detecting PMPs. However, studies with larger sample size are needed to validate our results.

Upfront Combined Hydroxyurea and Imatinib Versus Imatinib Monotherapy in Newly Diagnosed Chronic Phase Chronic Myeloid Leukaemia Patients With Reference to Early Molecular Response: A Randomized Controlled Trial

Rituparna Chetia, Arkapal Bandyopadhyay, Anamika Bakliwal, Sudeep Vaniyath, Debranjani Chattopadhyay, Ashok Rajoreya, Uttam Kumar Nath

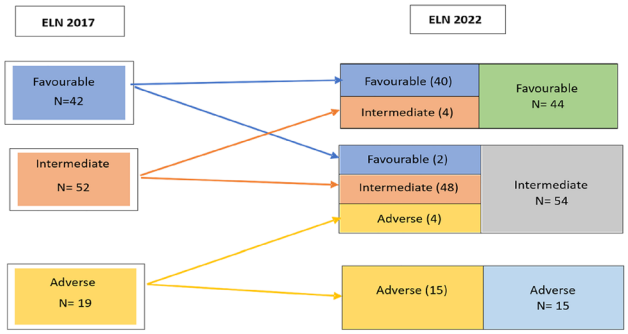
Introduction: Tyrosine kinase inhibitors like imatinib have become the standard therapy for chronic phase CML (CML-CP). Role of hydroxyurea, a DNA synthesis inhibitor has been less explored in CML-CP.
Aims & Objectives: The present study is conducted to compare the efficacy and safety of hydroxyurea & imatinib combination versus Imatinib monotherapy for upfront treatment of newly diagnosed CML-CP patients with reference to early molecular response.
Materials & Methods: The present randomised controlled trial was conducted in 90 newly diagnosed Chronic Myeloid Leukaemia patients in chronic phase (CML-CP), age ≥ 18 years and of either sex. Patients were randomised to receive structured dose of Hydroxyurea with Imatinib versus Imatinib alone as treatment. Patients were subsequently followed up every 2 weeks in OPD and after completion of 3 months of treatment. Blood sample was collected and quantitative real-time PCR for BCR-ABL1 (international scale; I.S.) was performed for assessment of early molecular response in both groups at 3 months. The safety evaluation of the two treatment arms were also compared.
Result: Median age in Imatinib + HU arm was 36 (IQR: 30–45) years whereas in Imatinib monotherapy arm was 38 (IQR: 31–47), majority were males. The most common symptom (62%) was fatigue and presenting sign was splenomegaly (89%). In 57 (63%) patients, cytogenetics showed presence of characteristic t(9;22) (q34;q11) chromosome. Majority of patients belonged to Intermediate risk group of Sokal score. Overall, 68 (76%) of study patients achieved early molecular response at 3 months of treatment and there was no statistically significant difference between two treatment groups (p = 0.53). The most common hematological toxicity was anemia and

non hematological toxicity was nausea and vomiting. There was no statistically significant difference between the two groups. (p = 0.08). **Conclusions**: Addition of hydroxyurea to imatinib in the present study was not found to significantly improve the haematological response or early molecular response (EMR). However, long-term studies with a larger sample size with structured dose of Hydroxyurea can be undertaken as a continuation to assess treatment outcomes.

Validation of ELN 2022 Risk Stratification System for Acute Myeloid Leukemia Patients: in Comparison with ELN 2017 from a Tertiary Care Centre

Ramesh Balasubramanian, Vandana Arya, NitinGupta, Jyoti Kotwal

Introduction: The risk stratification of Acute Myeloid leukemia (AML) by cytogenetics and molecular mutations has been dynamic and numerous changes have occurred from ELN 2017 to 2022. The major change was the addition of FLT3-ITD allelic ratio (AR) in ELN 2017. Both NPM1 and FLT3-ITD mutated with low allelic burden (AR < 0.5) were assigned the favourable risk group, whereas those with NPM1 mutation and FLT3-ITD high allelic burden (AR > 0.5) were assigned the intermediate risk category. ELN 2022 has removed AR and assigned all FLT3-ITD mutated to intermediate risk. NGS plays a crucial role in establishing the risk categories in ELN 2022.
Aims & Objectives: To validate ELN 2022 risk stratification in AML patients with cytogenetics and molecular mutations and comparing it with ELN 2017.
Materials & Methods: All AML patients presented to our hospital in past 3 years were diagnosed by bone marrow aspiration, biopsy and flowcytometry. Karyotyping and AML-PCR panel for NPM1, FLT3 ITD, AML::ETO, Inv(16) were done in all patients. FLT3-ITD allelic ratio was calculated by fragment analysis. NGS was done in selected patients due to financial constraints.
Result: Total of 113 AML patients were included. 60% (60/100) cytogenetically normal. Clinically significant mutations were detected in 56.7% (64/113). NPM1 and FLT3 ITD were the predominant mutations, irrespective of their cytogenetics. 3.5% (4/113) cases with NPM1 and low AR FLT3-ITD mutation were assigned favourable risk group of ELN 2017. According to ELN 2022, they were shifted to Intermediate risk group. All patients were treated with either 7 + 3 induction or Azacytidine according to their fitness. AlloHSCT was done in intermediate and adverse risk patients who are fit. Survival analysis did not show significant difference amongst ELN 2022 risk groups. There was no statistically significant improvement in 2 year OS & DFS of low AR cases designated to favourable group in ELN 2017. The survival of all FLT3-ITD patients were low, irrespective of AR.
Conclusions: The survival of all FLT3-ITD patients were low, irrespective of AR. Thus, elimination of AR by ELN 2022 seems to be valid, though large scale study is required to conclude this. NGS for all patients, as emphasized by ELN 2022 is practically impossible in resource constrained countries like India.



Randomized Controlled Study to Compare Toxicity Profile of Dexamethasone Versus Prednisolone in Induction Phase Chemotherapy in Pediatric & Adolescent ALL

Karthik, Kanimozhi, Sashikant Singh, Jhasaketan Nayak, Jasmine Porwal, Subhajit Hajra, Gaurav Dhingra, Puneet Dhamija, Harish Chandra, Uttam Kumar Nath

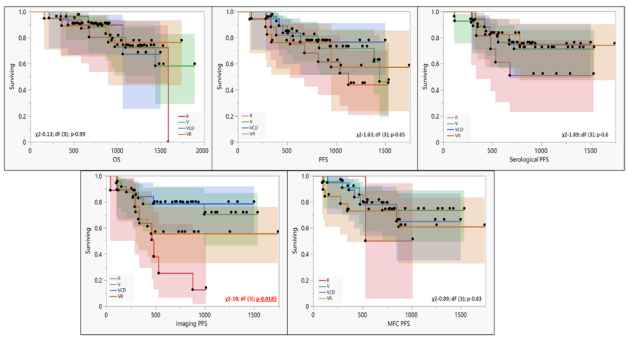
Introduction: Acute lymphoblastic leukemia (ALL) is the commonest childhood cancer. With the evolution of minimal residual disease (MRD)-adapted intensive pediatric treatment regimens in children and Adolescent & young Adult (AYA) ALL, survival rates have improved significantly. Multiple randomized studies compared the efficacy of dexamethasone & prednisolone in childhood ALL and showed dexamethasone had a superior response in high-risk ALL and decreased cumulative incidence of relapse, but at the cost of increased toxicity.
Aims & Objectives: To compare the adverse events (AE) in the two treatment groups (dexamethasone vs prednisolone) in induction therapy phase IA of ALL BFM 2009 protocol.
Materials & Methods: The present randomized controlled study enrolled newly diagnosed ALL patients in age group of 1–25 years at AIIMS Rishikesh between April 2021 & July 2022 after obtaining informed consent & ethical approval. Patients were randomized to receive either Dexamethasone 10 mg/m2/day administered intravenously on days 1–14 or Prednisolone 60 mg/m2/day per orally on days 1–28 during Induction Phase IA of modified ALL BFM 2009 regimen. Steroid dose was tapered off over next 7 days in both groups. Patients of infantile ALL (age < 1 year), lymphoblastic lymphoma (LBL), & patients who had already received steroid or any chemotherapy prior to enrollment in the study were excluded. The adverse events of all grade, and grade 3–4 as per CTCAE version-5 were compared in the two treatment groups.
Result: The differences in incidence of common steroid related AE's namely gastritis, proximal myopathy, hypokalemia, febrile neutropenia/sepsis, enterocolitis/typhlitis, hyperglycemia, hypertension, invasive fungal infection and septic shock were not statistically significant in the two steroid groups. There were seven deaths in induction [Pred: 2/7; Dexa: 5/7], three were not in remission, two had MDR-bacterial infection, two had invasive fungal infection (pulmonary aspergillosis &mucormycosis), and one had COVID-19 and was not statistically significant in the treatment groups.
Conclusions: In the present single-center experience, the toxicity profile of dexamethasone & prednisolone used in induction phase IA of ALL BFM 2009 protocol in pediatric& adolescent ALL were comparable, with no statistically significant increase in steroid-related adverse events in the dexamethasone group.

Adverse events	Steroids	Day 8 Any grade (n)	Day 8 Grade 3-4 (n)	Day 15 Any grade (n)	Day 15 grade 3-4 (n)	Day 33 Any grade (n)	Day 33 Grade 3-4 (n)
Allergy/Anaphylaxis	Prednisolone	4	0	0	0	0	0
	Dexamethasone	0	0		0	0	0
Anemia	Prednisolone	14	9	18	10	14	6
	Dexamethasone	20	12	20	13	13	5
Avascular necrosis of bone	Prednisolone	0	0	0	0	0	0
	Dexamethasone	0	0	0	0	0	0
Hyperbilirubinemia	Prednisolone	4	0	2	0	1	0
	Dexamethasone	2	0	1	0	1	0
Bleeding	Prednisolone	2	0	2	1	1	0
	Dexamethasone	5	1	2	2	0	0
Enterocolitis & Typhlitis	Prednisolone	2	1	6	1	3	1
	Dexamethasone	2	1	7	2	1	0
Febrile neutropenia / Sepsis	Prednisolone	4	4	8	8	3	2
	Dexamethasone	5	5	8	7	1	1
Gastritis	Prednisolone	17	0	9	0	3	0
	Dexamethasone	17	1	8	1	1	0
Headache	Prednisolone	6	0	0	0	0	0
	Dexamethasone	11	0	0	0	0	0
Transaminitis	Prednisolone	3	2	3	1	2	0
	Dexamethasone	4	2	5	2	3	1
Hyperglycemia	Prednisolone	6	1	3	0	1	0
	Dexamethasone	4	1	1	1	0	0
Hypophosphatemia	Prednisolone	8	0	1	0	0	0
	Dexamethasone	2	1	3	1	0	0
Hypertension	Prednisolone	5	1	5	0	2	0
	Dexamethasone	4	0	4	1	0	0
Tumor lysis syndrome	Prednisolone	1	0	0	0	0	0
	Dexamethasone	1	0	0	0	0	0
Hypofibrinogenemia	Prednisolone	12	3	15	5	12	5
	Dexamethasone	2	1	7	2	8	1
Hypokalemia	Prednisolone	3	0	5	1	4	2
	Dexamethasone	4	1	9	1	5	0
Invasive fungal infections	Prednisolone	0	0	2	2	2	2
	Dexamethasone	2	2	2	2	0	0
Lymphopenia	Prednisolone	9	7	14	6	2	0
	Dexamethasone	11	0	14	4	4	0
Myopathy	Prednisolone	17	0	14	1	6	0
	Dexamethasone	16	1	13	1	6	1
Nausea/vomiting	Prednisolone	12	0	9	0	2	0
	Dexamethasone	15	1	11	1	2	0
Acute pancreatitis	Prednisolone	0	0	0	0	1	1
	Dexamethasone	0	0	0	0	0	0
Neutropenia	Prednisolone	12	9	12	10	2	2
	Dexamethasone	21	20	17	15	4	0
Thrombocytopenia	Prednisolone	12	10	10	7	4	2
	Dexamethasone	18	16	15	9	4	4
Peripheral Neuropathy	Prednisolone	0	0	6	0	3	1
	Dexamethasone	0	0	4	0	0	0
Psychosis	Prednisolone	0	0	2	0	1	0
	Dexamethasone	0	0	1	0	0	0
Thrombosis (Central venous catheter-related)	Prednisolone	1	1	1	1	0	0
	Dexamethasone	0	0	0	0	0	0
GI bleeding	Prednisolone	0	0	0	0	0	0
	Dexamethasone	0	0	0	0	0	0
COVID 19 infection	Prednisolone	0	0	0	0	0	0
	Dexamethasone	0	0	1	1	0	0
Septic shock	Prednisolone	1	1	0	0	0	0
	Dexamethasone	1	1	3	3	0	0

Indian Multicenter Phase Ii Randomised Controlled Study Comparing Post Stem Cell Maintenance Regimen for Newly Diagnosed Multiple Myeloma (Impose Bortecon Study)

Uday Yanamandra, Rajan Kapoor, Suman Pramanik, Satyaranjan Das, Ankur Ahuja, Tathagata Chatterjee, Harshit Khurana,Rajiv Kumar, Kundan Mishra, Sanjeevan Sharma, Velu Nair

Introduction: Autologous stem cell transplant (ASCT) remains the backbone therapeutic modality with the highest progression-free survival (PFS) and overall survival (OS) benefit even in the era of the novel agents in newly diagnosed multiple myeloma (NDMM). The survival post-transplant can be prolonged using maintenance therapies. The regimen with maximum benefit is still debated, with bortezomib showing PFS benefit even in the high-risk myeloma.
Aims & Objectives: This randomized phase II trial is aimed at studying the efficacy (as measured by overall survival (OS), progression-free survival (PFS)), and safety of post-ASCT different maintenance regimens in patients with NDMM.
Materials & Methods: Multicentric open-label interventional study with randomized allocation, parallel assignment, with intention-to-treat analysis. Recruitment was prospective starting 01 Jan 2017, including all NDMM patients eligible for the study. Remission status was evaluated at D100 and every 6 months for 2y post-ASCT, including MRD analysis by multicolor flow cytometry (MFC) and PET/CT. The four arms included (Arm-A) bortezomib alone (V), (Arm-B) bortezomib in combination with cyclophosphamide and dexamethasone (VCD), (Arm-C) bortezomib in combination with lenalidomide (VR), and (Arm-D) Lenalidomide starting D100 till 2y post-ASCT. Adverse events with CTACE grade < 2 were defined as non-serious and the rest as serious. JMP ver. 13 was used for statistical analysis and $p < 0.05$ was considered significant. Kaplan Meier statistics was used for survival analysis.
Result: A total of 123 patients have enrolled of which 92 patients completed the study protocol and the rest 31 patients were excluded because of protocol deviation due to the COVID pandemic. The median age of the study population was 54.5y (35-76y) with a male preponderance (67%). There was no statistically significant difference between the four arms on the log-rank test in the OS (p-0.99), clinical PFS (p-0.65), biochemical PFS (p-0.6), or MFC-based PFS (p-0.83). There was a statistically significant difference between the four arms on the log-rank test (p-0.0185) on PET/CT-based PFS (PFS being in a descending order VCD > V > VR > R regimen). The all-cause mortality of the study participants was 19.57% (n-18) and the difference in deaths among the various groups was not statistically significant (p-0.85). The tolerability, serious and non-serious adverse were significantly higher amongst Arm D patients.
Conclusions: We conclude that there was no difference in OS between the different regimens. Patients on Lenalidomide-only therapy had significantly inferior Imaging-PFS.



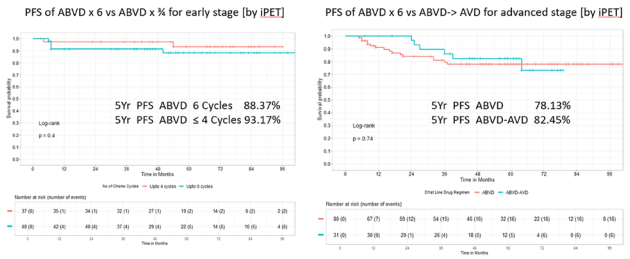
Hodgkin Lymphoma: Experience with Pet Guided De-Escalation of Therapy in Real World

Akshay Lahoti, Susmita Dasgupta, Bibhas Chakraborty, Pranita Mishra, Rimpa Basu Achari, Arijit Nag, Rizwan Javed, Saurabh Bhave, Jeevan Kumar, Vivek Radhakrishnan, Mammen Chandy, Ramesh Nimmagadda, Reena Nair

Introduction: Acceptance of de-escalating therapy in Hodgkin Lymphoma (HL) in the developing world has been slow due to limited access and high cost of PET scans and the physician acceptance.
Aims & Objectives: To study the clinical characteristics, treatment patterns and 5- year outcomes of HL treated in a tertiary cancer center with abbreviated cycles of chemotherapy in early stage and omission of bleomycin in advanced stage in patients who achieve complete metabolic response to standard Adriamycin, bleomycin, vinblastine and dacarbazine (ABVD).
Materials & Methods: 294 HL ($\geq$ 18 years) were entered in the Onco-Collect data base of patients receiving 1st line treatment from May 2011 to December 2019, followed till July 2022. Demography, Clinical features, staging, prognostic stratification, first line treatment response and outcomes of 269 classical HL (cHL) patients treated at a tertiary center have been analyzed. A PET directed approach was followed for 196 patients receiving standard ABVD therapy. Early stage (1 and 2) patients received 4 or 6 cycles and advanced stage (3 and 4) patients received ABVD $\times$ 6 cycles or ABVD $\times$ 2 cycles followed by AVD in patients who were in complete remission after 2 cycles. The outcomes of these patients were compared to those who received Standard 6 cycles of ABVD.
Result: cHL formed 91% of the HL cohort receiving 1st line treatment at our Institute. 251 (92%) patients received standard ABVD

chemotherapy. The median age was 37 years at presentation and age distribution was 54%, 39% and 7% respectively for < 40 years, between 40 to 64 years and ≥ 65 years of age. Male to female ratio was 2.02:1. 38% patients presented in early stage [I & II], 62% in late stage [III & IV].
The OR (CR + PR + SD) rate was 94%, and progression occurred in 5%. With a median follow-up of 48 months, the PFS at 5 years is 90% for early stage and 77.25% for late stage HL.

48 patients with early-stage disease receiving 6 cycles had similar outcomes at 5 years compared to 36 patients receiving 4 cycles ± radiotherapy. The 5 year PFS was 88% vs 93% (p = 0.4). In late stage disease, in 31 patients bleomycin was omitted after 2 cycles as were in PET CR and 80 patients received 6 cycles of ABVD, the 5 year PFS was 82% vs 78% (p = 0.74).
Conclusions: HL presents at a median age of 37 years in the adults ≥ 18 years and the bimodal peak is not seen in our population. The OR to treatment is 94% for standard ABVD therapy, with CR in 83% patients. Our initial experience with de-escalation of therapy have yielded encouraging results in the REAL world setting.



Standarization of Digital Droplet PCR (DDPCR) for t(8;21) RUNX1::RUNX1T1 MRD Monitoring In Acute Myeloid Luekemia

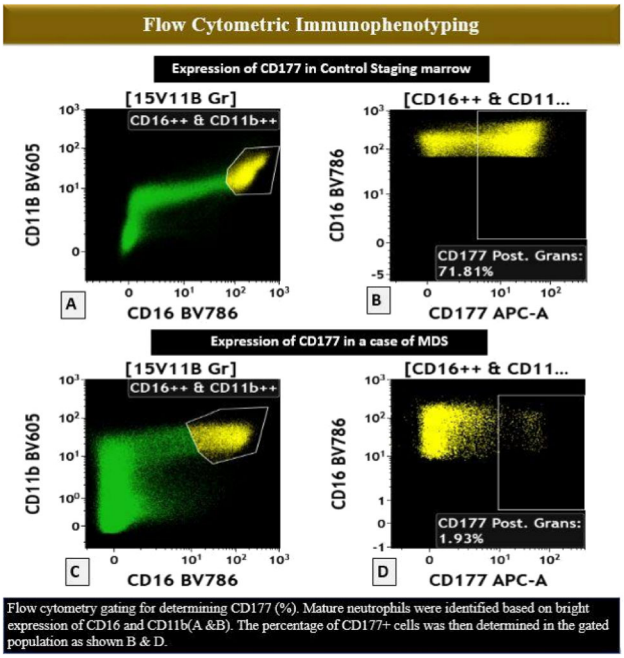
Poonam Das, Sourav Sarma Choudhury, Indranil Dey, Rakesh Demde, Niharendu Ghara, Arijit Nag, Jeevan Kumar, Saurabh Jayant Bhave, Reena Nair, Asish Rath, Mayur Parihar, Sushant S Vinarkar, Deepak Kumar Mishra

Introduction: Acute Myeloid leukemia (AML) with t(8;21)(q22;q22) generally shows maturation arrest in the myeloid lineage. AML represents around 20% of cases of acute leukemia in childhood. An optimized MRD monitoring methods is required which can help to intervene before relapse. ddPCR can be of the more sensitive and precise technique for detection because it provides absolute quantification without any standard Curves.
Aims & Objectives: Standardization and validation of ddPCR based quantification of RUNX1::RUNX1T1 transcript MRD monitoring in RUNX1::RUNX1T1 positive AML patients.
Materials & Methods: In this study the RUNX1–RUNX1T1assay was performed on BIO-RAD QX200 ddPCR platform with ABL as housekeeping gene. The primers,probes were selected from published literature, which were procured from BIO-RAD.Theoptimization of the ddPCR parameters was done by running the assay at different annealing temperature, RAMP rate and PCR cycles. The optimized parameters resulted in high fluoresce signal which helped in better resolution of positive and negative droplets.
For validation of the assay, 12 known patients of AML with t(8:21) diagnosedon FISH /RT-Nested PCR and known commercial fusion control (SeraSeq Myeloid fusion RNA Mix) were used. For these 12patients, samples at various time points (Diagnosis, Post Induction, and Post Consolidation) were processed. To optimize background false positive rate 10 healthy samples were run as negative sample. The RNA was extracted using Qiasymphony Instrument (Qiagen) as per manufacturer instruction and c-DNA conversion was done from TRUPCR kit. The plasmid containing the fusion gene sequences were constructed (expressing RUNX1::RUNX1T1) to detect the lower limit of detection of the assay.
Result: Out of 12 patients, 58.3% were pediatric AML (mean age = 8 yrs) and 41.7% were adult AML (mean age = 39 yrs). Optimization of PCR cycles (40cycles to 72cycles) showed sevenfold increases in fluorescence intensity of positive droplets as compared to negative droplets. At diagnosis RUNX1::RUNX1T1 positive sample showed 100% concordance, while follow up sample showed discordance of 8.
Conclusions: Droplet digital PCR is a reliable technique to monitor MRD in AML to detect early relapse and has the potential to become robust technique for accurately quantifying RUNX1::RUNX1T1.

Clinical Utility of CD177 in the Diagnosis of Myelodysplastic Syndrome

Denna Sarah Prabhin, Avinash Gupta, Aswathy Anilkumar, Jagruti Patil, Sitaram Ghogale, Nilesh Deshpande, Badrinath Yajamanam, Karishma Girase, Harshini Sriram, Shweta Rajpal, Gaurav Chatterjee, Nikhil Patkar, Dhanlaxmi Shetty, Sumeet Gujral, P. G. Subramanian, Prashant Tembhare

Introduction: CD177, also known as the NB1 or HBA-2 antigen, is a glycosylphosphatidylinositol (GPI)-linked cell surface antigen expressed exclusively on neutrophils. Neutrophils show a bimodal expression pattern of CD177. Both the fraction and expression level of CD177 increase during neutrophil maturation with approximately 45% to 65% of mature segmented neutrophils expressing the protein. Multicolour flow cytometry(MFC) is a rapid and useful tool in the diagnosis of myelodysplastic syndrome(MDS).
Aims & Objectives: To study the downregulation of CD177 in CD11b strong(+) and CD16 strong(+) granulocytes(neutrophils) as an additional parameter for the MFC diagnosis of MDS.
Materials & Methods: We studied the expression of CD177(AP-C,REA258) on mature neutrophils(CD16++ & CD11b + +) in bone marrow samples of MDS(n = 30) and non-myeloid neoplasm(n = 50) (including staging marrows) cases using a 10–13 color panel. Cells were acquired on Cytoflex (Beckman Coulter) and data was analyzed using Kaluza software v2.1.
Result: CD177 expression was studied in a total of eighty cases (fifty non- myeloid & thirty MDS). Median(range) of CD177(+) neutrophils in non-myeloid neoplasms and in MDS cases were 79.18 (23.48%-99.6%) and 22.84(1.6%-96.6%) respectively. We observed pathogenic loss of CD177 and determined cut-off of < 36.
Conclusions: Downregulation/loss of CD177 in mature granulocytes (< 36.0%) is a distinct feature of dysgranulopoiesis and valuable addition to immunophenotypic abnormalities of MDS. Hence, it can be incorporated into MFC diagnosis and scoring systems for MDS.



Correlation Between Flow Cytometric Expression of Crlf2 and Fish Rearrangement of CRLF2: A Study of 30 Cases from Tertiary Cancer Centre in North East

Karthik R, Aaishwarya Dhabe, Sipra Rani Patel, Rakesh Demde, Subhajit Brahma, Sambhunath Banerjee, Niharendu Ghara, Arpita Bhattacharya, Arijit Nag, Jeevan Kumar, Saurabh Jayant Bhave, Reena Nair, Sushant Vinarkar, Asish Rath, Deepak Kumar Mishra, Mayur P

Introduction: Rearrangements of the Cytokine receptor-like factor 2 (*CRLF2*) gene account for almost 50% of the Ph like B cell precursor acute lymphoblastic leukaemia (BCP-ALL). *CRLF2* gene rearrangements are often associated with overexpression of the protein and can be detected by multi-parametric flow cytometry (MFC). We report correlation of expression of CRLF2 protein with gene rearrangements identified using fluorescent in situ hybridisation (FISH).
Aims & Objectives: To evaluate the concordance between flowcytometry expression of CRLF2 and cytogenetic findings suggestive of *CRLF2* rearrangement.
Materials & Methods: This was a retrospective study for a period of 7.5 years from January 2015 to June 2022. The B-ALL cases which showed CRLF2 expression on flow cytometric analysis using BV510 mouse antihuman TSLP receptor (BD horizonTM) were included in this study. The retrieved cases were further subjected to conventional karyotyping and Interphase FISH analysis using *CRLF2* gene break-apart probe (ZytoVision GmbH, Bremerhaven, Germany).
Result: Thirty patients were identified with expression of CRLF2 on flowcytometry. Male to female ratio (M:F) was 1.5:1. *CRLF2* gene rearrangement was identified on FISH analysis in 10 patients (33.3%), with nine of these showing expression > 50% on flowcytometry. The majority of these (eight patients) were identified with *CRLF2::P2RY8* fusion and two showed *CRLF2::IgH* fusion. Of the 20 patients negative for *CRLF2* rearrangement by FISH analysis, 15 showed high [removed] > 70%) and five showed low [removed] < 20 > Karyotype analysis of the 20 *CRLF2* rearrangement negative patients revealed high hyperdiploidy in six patients, *BCR::ABL1* fusion in two, *cMYC, E2A, KMT2A* rearrangement in one patient each. One patient each belonged to near triploidy, near tetraploidy and complex karyotype category. Six (30%) cases were classified in the B- others group.
Conclusions: CRLF2 expression on flow cytometry may be indicative but not always confirmatory of*CRLF2*gene rearrangement. Cytogenetic analysis is required to confirm rearrangement and rule out other possibilities leading to increased expression of the CRLF2 protein. Increase copy number of sex chromosomes as seen in high hyperdiploidy, near triploidy, near tetraploidy also result in increased expression of CRLF2 on flowcytometry.

Prospective Analysis of the Genomic Landscape of 'B-Other ALL' and Its Impact on Clinical Outcome: Data from a Single Tertiary Cancer Care Centre in India

Purvi Mohanty, Dhanlaxmi Shetty, Kruti Chaubal, Bhagyashree Khot, Hemani Jain, Prashant Tembhare, Nikhil Patkar, P.G. Subramaniam, Chetan Dhamne, Nirmalya Moulik, Hasmukh Jain, Jayashree Thorat, Lingaraj Nayak, Manju Sengar, Akanksha Chichra

Introduction: ~ 30% of B-ALL patients remain unclassified at the genetic level and are assigned intermediate risk, 'B-other-ALL'. Genomic studies have led to the discovery of novel genetic drivers, classifying patients negative for recurrent cytogenetic abnormalities (B-Other ALL) into 'B-ALL with other defined genetic abnormalities' and 'Ph-like ALL'; providing definitive prognostic information for therapeutic intervention leading to improved outcomes. The characterization of 'B-Other ALL' and 'Ph-like ALL' genome is still in nascent stage in Indian settings with no published data till date.
Aims & Objectives: The aim of the study was to determine the prevalence of 'B-ALL with other defined genetic abnormalities' and 'Ph-like ALL' subgroups in Indian population and correlate with post induction/consolidation minimal residual disease (MRD) and 2-year overall survival.
Materials & Methods: A two tier diagnostic algorithm employing Fluorescence In-Situ Hybridisation (FISH) as a first-hand approach to detect 'B-ALL with other defined genetic rearrangements' (and 'Ph-like ALL' aberrations followed by targeted next generation sequencing was developed to classify a cohort of n = 765, 'B-Other ALL' diagnosed between January 2019 and March 2022. Flow cytometry based MRD assessment was performed at the end-of-induction/consolidation to assess response to therapy and overall survival was estimated using the Kaplan–Meier method.
Result: The study cohort comprised of 38.7% (765 of 1977 B-ALL) 'B-Other ALL' patients including 40% patients < 11 > 40 yrs of age. We identified 'Ph-like ALL' aberrations in 18% (138/765) and 'B-ALL with other defined genetic abnormalities' in 20.7% (158/765) cases. ABL-Class (3.8%) Ph-like abnormalities included ABL1-r (1.2%), ABL2-r (0.3%), PDGFRβ-r (2.1%) and CSF1R-r (0.3%); JAK-Class (4.8%) abnormalities identified were CRLF2-r (2.9%), JAK2-r (1.7%) and EPOR-r (0.3%) while other aberrations included *IKZF1* deletions (7.8%) and ETV6-r (1.3%). 'B-ALL with other defined genetic abnormalities' included; PAX5-r (6.7%), MEF2D-r (3%), ZNF384-r (2.6%) and IGH::DUX4 (3.1%). Novel fusions identified were ETV6::VWC2, UBR4::FGFR1, EBF1::IGK, PAX5::ANKRD12 and PAX5::GEMIN8. Post-induction MRD was positive (> 0.01%) in 40.3% 'Ph-like ALL' and 10.2% B-ALL with other genetic abnormalities patients with 9% overall death rate of the study cohort receiving standard treatment.
Conclusions: Our testing strategy is a practical tool that can be used to identify 'Ph-like ALL' and 'B-Other genetic abnormalities' in a timely manner, with potential to guide treatment decisions. Overall the study provides valuable insights into the genomic landscape of 'B-Other ALL' in India associated with distinct prognostic features.

Validation of NGS Based RNA Fusion Sequencing in Myeloid Neoplasm: A Single Center Experience

Kallol Saha, Saheli Banerjee, DebajaniNathi, Indranil Dey, Rakesh Demde, Arijit Nag, Jeevan Kumar, Saurabh Jayant Bhave, Reena Nair, Asish Rath, Mayur Parihar, Sushant S Vinarkar, Deepak Kumar Mishra

Introduction: The importance of recurrent gene fusions in the diagnosis, risk stratification, treatment planning and response assessment (MRD) in myeloid neoplasmas has been well established. With the recent development of techniques such as Next Generation RNA sequencing the detection of fusions has become relatively easy and systematic.
Aims & Objectives: Clinical validation of NGS based RNA fusion detection panel at our center.
Materials & Methods: The validation exercise was carried out using the Oncomine Myeloid Panel TM. The validation assay was divided into four parts which comprised of analytical sensitivity, specificity, precision and limit of detection. The analytical sensitivity of the assay was verified using 10 known fusion positive cases detected on either on RT-PCR/FISH/Karyotyping. Specificity of the assay was determined using normal controls from healthy adults (n = 4). The validation of uncommon fusions and precision of the assay was performed with Seracare-Myeloid Fusion RNA Mix. The limit of detection was performed with known standards of 1 copy and 10 copies of BCR-ABL(e14a2-ERM628-Sigma) fusion controls. The validation was carried out in the Ion Torrent PGM NGS platform(4 runs on 318 chip) and Genestudio S5 NGS platform(2 runs on 520/530 chip each). The bioinformatics pipeline involved the Ion reporter custom filter (318/530 Oncomine Myeloid Fusion single sample).
Result: Sensitivity of the assay was at 100% as no false negative (capacity to detect true sequence variants) cases were found. The specificity of the assay was 100% as no false positive samples were detected. The precision of the assay corroborated with the commercially available control as all the eight fusion types were satisfactorily detected. The mean reads per sample in Ion Torrent PGM run is 160350, mean reads for the S5 530 chip was 200,451 and 520 chip was 184,751. The limit of detection with the BCR-ABL1 standard was noted as 1 copy.
Conclusions: We performed validation exercises to detect RNA fusion transcripts by NGS utilising the Oncomine Myeloid Panel and it revealed 100% sensitivity and specificity with good precision and LOD of 1 copy. A well validated NGS based RNA sequencing assay can be instrumental in the reliable, accurate and cost effective detection of RNA fusions in myeloid malignancies.

Modulating Effects of Alpha Globin Gene Mutations in Defining the Phenotypes of IVS1-5(CC) Beta Thalassemia Patients

Jyoti Shaw, Sunistha Bhattacharyya, Anjumana Khatun, Maitreyee Bhattacharyya

Introduction: Severity of Beta thalassemia ranges from mild to severe anemia. Patients with severe anemia become completely dependent on regular blood transfusions for sustaining life. This wide difference in phenotypic severity is considered to lie on the genetic makeup of globin genes. It is already shown that co-inheritance of alpha globin deletion in beta thalassemic modifies the phenotypic severity of beta thalassemia. However there is no study on the effect of alpha triplication.
Aims & Objectives: Aim is to study the effect of alpha globin gene mutations on the phenotypes of IVS1-5(G > C) homozygous Beta thalassemia patients. The objective was to investigate alpha triplication and alpha deletion in IVS1-5(G > C) homozygous beta thalassemia patients.
Materials & Methods: The study population comprised of Beta thalassemia patients who attended the Thalassemia OPD of IHTM, MCH, Kolkata. After obtaining clinical history and informed written consent, peripheral blood samples were collected from the patients. CH and HPLC were done for all the patients. Detection of IVS1-5(G > C) point mutation was done by ARMS-PCR and Alpha globin gene mutations were detected by GAP–PCR method.
Result: 676 thalassemia major patients were selected. Out of this 366 were confirmed as Beta thalassemia major. These 366 patients were subjected to mutation analysis and among them 121 were found to possess IVS1-5(G > C) mutation in homozygous state. These IVS1-5(CC) patients were further analyzed for presence of alpha globin gene mutations. Out of 121 cases, 15 patients had alpha 3.7 triplication (ααα 3.7anti) and 24 had alpha 3.7 deletion (-α3.7) mutation. Phenotypic severity was compared among three sub-groups with alpha genotype of 'ααα 3.7anti'; '- α3.7/αα' and 'αα/αα'. The results were shown in the table below. Mean age of first transfusion was lower in ααα 3.7anti group as compared to others. Even serum ferritin and spleen size are higher in this group as compared to other groups. Baseline Hb is lowest in this group as compared to others.
Conclusions: The results shows that beta thalassemia major with co-inheritance of ααα 3.7anti have more severe phenotypes. However, this is not depicted in transfusion frequency as 46% of ααα 3.7anti group underwent splenectomy. And after splenectomy, transfusion requirement decreases. Hence, co-inheritance of ααα 3.7anti further increases the alpha:non-alpha chain ratio. These excess alpha chains are the main determinants of severity in beta thalassemia.

Table : Phenotypic comparison of IVS1-5 (CC) beta thal with co-inheritance of alpha mutation.

Clinical Features	$\alpha\alpha\alpha^{3.7anti}$ (n=15)	$-\alpha^{3.7}/\alpha\alpha$ (n=24)	αα/αα (n=82)
Mean age_diagnosis (yr)	4.85	6.05	3.5
Mean age_1st BT (months)	8.67	21.6875	13.49
Baseline Hb	6.84	7.02	6.98
Mean BT/yr	10.72	10.75	12.8
Mean serum ferritin	2640	1986	2554.12
Mean HbF	16	19.16	21.86
Spleen size	7	4.12	3.22
Liver size	2.83	3.27	2.96
*Splenectomised	7	3	10
*G.R.	6	7	24

'*' – denotes number of patients having the parameters

A Limited and Economical Genotyping Panel Unravels the Molecular Genetic Basis of Beta-Thalassemia Intermedia in a Majority of Cases: A Study on 256 Indian Patients

Namrata Singh, Jasbir Kaur Hira, Sanjeev Chhabra, Alka Rani Khadwal, AmitaTrehan, Deepak Bansal, Richa Jain, Pankaj Malhotra, Reena Das, Prashant Sharma

Introduction: β-thalassemia intermedia (β-TI) comprises a group of enigmatic disorders displaying widely divergent clinical phenotypes. Their heterogeneous presentations are uniquely explained by underlying HBB gene variants (primary modifiers) along with co-inherited secondary modifiers like α-globin gene dosage and modulators of HbF levels.
Aims & Objectives: To study the molecular spectrum of a large north Indian β-TI cohort using PCR/sequencing-based testing.
Materials & Methods: Cases diagnosed clinically as β-TI (n = 256) between 2007–2022 underwent testing for HBB variants using amplification-refractory mutation system-PCR and/or direct-DNA sequencing. Eight common α-globin gene deletions were tested by

multiplex gap-PCR, supernumerary α-globin genes by gap-PCR, and Xmn1Gγ-polymorphism (-158Gγ C > T) using PCR–RFLP.
Result: Average age at diagnosis was 15 years (range 2 months to 63 years). Among HBB variants, IVS1-5 G > C (HBB:c.92 + 5 G > C) was commonest (20.6% alleles). The five common Indian β-thalassemia mutations [HBB:c.92 + 5G > C, HBB:c.92 + 1G > T/A, HBB:c.126_129delCTTT, HBB:c.27_28insG and NG_000007.3:g.71609_72227del619] comprised 53.5% alleles (vis-a-vis > 90% in transfusion-dependent β-thalassemia historically). A β++ promoter -88C > T (HBB: c.-138C > T) was found in 17.1% alleles. Twenty-five patients (9.7%) were homozygous/compound heterozygous for β+/β++ variants. Overall, 71 cases showed co-inherited α-thalassemia: 25.7% had -α3.7 (64 heterozygotes, 2 homozygotes) and 1.9% had -α4.2 deletion (4 heterozygotes, 1 homozygote). Supernumerary α-globin genes (triplications, quadruplications etc.) were found in 10.5% patients: ααα anti3.7 in 24 and αααanti4.2 in three cases. Xmn1Gγ status was+/+ in 39/256 (15.2%) and ± in 68/256 (26.5%). TI phenotype in 26 (10.1%) β-thalassemia traits (with a single HBB mutation) was explained by supernumerary α-globin genes. Among 91 patients homozygous/compound heterozygous for severe HBB mutations, 37 (40.6%) had co-inherited α-thalassemia and 30 (32.9%) were Xmn1Gγ+/+. Only 8 (8.8%) patients with severe HBB mutations had neither α-thalassemia nor an Xmn1Gγ + allele, and thus remained unexplained.
Conclusions: A systematic test-panel-based genetic analysis of β-TI explains the clinical presentations in nearly 90% north Indian cases. Common genotypes include inheritance of milder HBB mutations, coinherited α-thalassemia (in homozygotes/compound heterozygotes for severe mutations) and the Xmn1Gγ+/+ genotype; or (in case of β-thalassemia traits), inheritance of excess α-globin genes.

Clinical Profile and Impact of Viral Infections (CMV, EBV & PARVOVIRUS) on Pediatric Hematoncology Patients

Purvaja Kubde, Swathi Krishna, Sneha Shinde, Dhara Shah, Trupti Dabhale, Vaibhav Chadhha, Purva Kanvinde, Ritika Khurana, Minnie Bodhanwala, Sangeeta Mudaliar

Introduction: Viral infections in haematological patients may result from reactivation of latent infection or from acquisition of new infections. Viruses particularly of concern are Herpes viruses (CMV, EBV) and Parvovirus B. Patients with haematological disorder can develop one of these viral infections at disease presentation or anytime during the treatment course.
Aims & Objectives: To study an impact of viral infections on hematoncology patients.
Materials & Methods: Patients with diagnosed or suspected underlying benign or malignant haematological conditions with prolonged fever or cytopenias, who underwent testing for EBV, CMV or Parvovirus infections by Polymerase chain reaction, between the period January 2020 to January 2022 were analyzed.
Result: Out of 165 patients who underwent testing, 38 patients (23%) tested positive with Parvovirus B in 23 (14%), EBV in 10 (6%) and CMV in 5 (3%).
Amongst patients with malignancy (47%) (7 ALL, 4 relapsed ALL, 4 AML, 2 HL,1 NHL) 11 patients had Parvovirus, 4 had CMV & 3 had EBV infection. In this cohort, 2 patients were in induction, 1 in consolidation, 12 were in maintenance, 1 patient with EBV infection developed NHL post liver transplant and 1 had CMV retinitis. Amongst 18 patients, 12 had refractory fever and cytopenias and 2 had HLH.

In non-malignant cohort (53%) (2 AIHA, 7 Aplastic anaemia, 3 sickle cell anemia,3 ITP, 2 HLH, 1 Fanconi anaemia, 1 hereditary spherocytosis,1 hemolytic anaemia under evaluation) 12 had Parvovirus, 1 had CMV and 7 had EBV infection. Out of 20 patients, 13 had viral infections at disease presentation & 7 developed it during course of treatment.

For Parvovirus infection, mean time of clearance of virus was 3 weeks. There was delay in chemotherapy in 8 patients. For patients with CMV infection, valganciclovir was administered for 6 weeks. In patients with HL & NHL with EBV infection, rituximab was given.
Conclusions: Viral infections impose a negative impact on hematoncology patients, increase financial burden and delays in treatment. Hence, screening for viral infections in hematoncology patients with unexplained cytopenia with prolonged fever and increased transfusion requirement is suggested.

Clinical Outcomes of Allogeneic Stem Cell Transplantation in Adolscents and Young Adults with Thalassemia Major: A Single Centre Experience

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Introduction: Data on allogeneic hematopoietic cell transplantation (Allo-HCT) for Thalassemia Major (TM) in adolescents and young adults (AYA) is scarce.
Aims & Objectives: To describe the clinical outcomes of Allo-HCT in AYA with TM.
Materials & Methods: Retrospective analysis using hospital records of all patients with TM who underwent Allo-HCT at our center from January 1994 to June 2022.
Result: Out of 771 Transplants for TM in our center, 142 were for patients of the age 13 and above (AYA). Median age was 15 (13–25) years and 84 (59.2%) were males. 3(2.1%), 13 (9.2%), and 126 (88.7%) transplants were for patients in Lucarelli Class I, II, and III, respectively. Of the 126 Class III patients, 94(66.2%) were classified as Class IIIHR (Age ≥ 7 years and liver size 5 ≥ cms). 124 patients underwent a matched related donor transplant (87.3%) and the remaining 18 were MUD transplants. Up to October 2009 the conditioning regimen was BuCy (32.1%).Stem cell source was bone marrow alone with which a high incidence of sinusoidal obstruction syndrome (SOS), graft rejection and treatment-related mortality (TRM) was noted. Hence the conditioning regimen was changed to to a combination of treosulfan, fludarabine and thiotepa. The stem cell source was changed to PBSC in class III patients from November 2010 due to concerns of early mixed chimerism and graft rejection with bone marrow stem cells. Median CD34 cell dose was 8.3 (2.1–23.9) × 106/kg. Cyclosporine and short course methotrexate was used as GVHD prophylaxis.
17 patients died before neutrophil engraftment of which 3 had primary graft failure. Of the remaining 125 patients who had neutrophil engraftment (median day + 16 (range 8–43)), acute GVHD was noted in 47 (33.1%) patients with 32(22%) having grade 3–4 GVHD (Steroid refractory in 19(13.4%) patients). 17(12%) patients had chronic GVHD and had 10 extensive GVHD per revised Seattle criteria. At last follow up (Median 631 days (range 0–8820)), the 2 or 3 year overall survival was 62%. Mortality was significantly increased in patients who received Busulfan based conditioning (p = 0.022), class IIIHR (0.035), aGVHD (p = 0.013) and extensive cGVHD (p = 0.004).
Conclusions: Allo-HCT for AYA with TM is associated with reasonable cure rates. Strategies to reduce GVHD and improve outcomes in ClassIII High-risk patients are required..

Assessment of Liver Fibrosis by Fibroscan as a Surrogate Marker in Beta Thalassemia Major Patients

Sukanya Priyadarshini Mohanty, Rabindra Kumar Jena, Sudha Sethy

Introduction: Iron overload (IO) is a major concern in Beta-thalassemia major (TM) patients who require lifelong blood transfusion to survive, consequences being significant liver fibrosis and ultimately cirrhosis. Therefore, monitoring of LIC (Liver Iron Concentration)nis of utmost importance to prevent life-threatening complications. Although Liver biopsy is the gold standard for estimating LIC, it has its own shortcomings such as invasiveness, sample variability because of uneven iron distribution, and interobserver variability. Magnetic resonance imaging (MRI) transverse relaxation time (T2*) is considered the method of choice for detecting IO in the liver, but is expensive, requires an expert radiologist for interpretation and not widely available. The need of the hour is a non-invasive, reliable, reproducible and affordable tool to detect the onset of fibrosis in these patients so as to intervene early and delay progression. Transient elastography (TE) is a valuable non-invasive technique of measuring liver stiffness and a reliable tool for predicting hepatic fibrosis in these patients.
Aims & Objectives: The role of TE in patients with b-thalassemia hasn't been extensively investigated. The present study aims to evaluate the role of TE in the assessment of hepatic fibrosis and its correlation with serum ferritin in 55 TM patients.
Materials & Methods: Cross-sectional assessment of hepatic fibrosis by TE was performed in 55 TM patients and their serum ferritin measured.
INCLUSION CRITERIA
1. Beta Thalassemia major (TM) patients.
2. Age > 10 years.
EXCLUSION CRITERIA
1. Diagnosed c/o other liver pathology (hepatitis B, hepatitis C, HIV, Wilson's disease, autoimmune hepatitis).
2. Paients with evidence of other cause of cirrhosis (imaging evidence, bilirubin > 5 mg/dL, ascites, encephalopathy, variceal bleed).
3. Age < 10 >
Result: Of 55 TM patients,33(60%) are male, 22(40%) are female. Patients were stratified for IO according to serum ferritin values as mild (< 1000 ng/ml), moderate(1000- < 2000 ng/ml),severe (2000- < 4000 ng/ml),very severe(> 4000 ng/ml). There are 3(5.45%),15(27.27%),33(60%),4(7.27%) patients in each group, respectively. The respective mean Liver Stiffness Measurement(LSM) values analysed in mild IO is 7.14 kPa(3.9 kPa-8.9 kPa),moderate IO is7.452 kPa(4.55 kPa-17.13 kPa),severe IO is 9.242 kPa(4.9 kPa-18.4 kPa) and very severe IO is 10.01 kPa(7.05 kPa-11.9 kPa).
Conclusions: Data analysis by SPSS Software is awaited.

Comparitive Study of Cellular Morphology of Bone Marrow Aspiration, Bone Marrow Imprint Smear and Bone Marrow Biopsy in Tertiary Care Centre

Priti Toppo, RK Nigam, Reeni Malik, Rita Saxena, Maneesh Sulya

Introduction: Hematological disorders encompass a broad spectrum of disorders affecting blood & bone marrow. Among a myriad of diagnostic tests that can be applied to the analysis of hematological diseases, bone marrow examination is one of the most valuable diagnostic tool. The Bone marrow aspirate and trephine biopsy specimens are complementary and when both are obtained, provide a comprehensive evaluation of the bone marrow.
Aims & Objectives: To compare the cellular morphology of Bone marrow aspiration and imprint smears in reaching the diagnosis of hematological disorders.
Materials & Methods: A total number of 60 cases in whom bone marrow examination was indicated were evaluated by Bone marrow aspiration(BMA), bone marrow imprint(BMI) and bone marrow biopsy(BMBx).
Result: The diagnosis of BMI correlated with the diagnosis of BMBx in 92% cases, which was higher than the value observed with BMA smears (75%). The spreading quality was better and cytological details were better appreciated in BMI as compared to BMA. All of these findings were reflected in the higher diagnostic accuracy of BMI than BMA.
Conclusions: BMI should be a standard practice for considering as an early and reliable diagnostic tool for evaluating bone marrow pathologies.
Diagnosis by bone marrow aspirate, bone marrow imprint and bone marrow biopsy.

DIAGNOSIS	BMA	BMI	BMBx
Granulomatous	1	3	3
Multiple myeloma	2	3	3
Megaloblastic	14	14	14
HLH	1	1	2
Reactive to infection	5	5	5
Aplastic anemia	0	0	11
Dimorphic	4	4	4
CML	5	5	5
Acute leukemia	5	5	5
Lymphoproliferative 50%	1	1	2
MDS	2	2	2
ITP	3	3	4

HLA Matching Profile Among Transplant Eligible Patients in Indian Scenerio

Surbhi Saxena, Uday Yanamandra, Suman Pramanik, Rajan Kapoor, Lavan Singh, Ajay Baranwal, Manisha Aggarwal, Satyaranjan Das, Ajay Sharma, Velu Nair

Introduction: Hematopoietic cell transplantation (HCT) can be a life-saving therapy for patients with genetic and acquired hematologic diseases. The outcomes of HCT depend on the HLA-match status, with the best results from fully matched donors. With the increasing use of haploidentical transplants in countries with one child policy, it is important to understand the demographics of the HLA match status in our settings to understand its utility in our country.
Aims & Objectives: In this study, we aimed to study the age- and gender-specific HLA-matching of transplant-eligible patients visiting the hematology department of a tertiary care center.
Materials & Methods: This is a retrospective study conducted in the hematology department of a tertiary care center in North India, conducted over a period of 14 years from 2004–2018. In this study, all transplant-eligible patients visiting the hematology department

were screened for HLA Matching using serological methods within the institute. The study included data regarding the number of relatives screened and average candidates matched per patient. The number of relatives screened per patient was further differentiated as per age-specific and gender-specific profiles. This study also includes the data as per average HLA matching score per relative screened for each patient. The data was descriptive in nature, all categorical variables were described as percentages and continuous variables as mean ± SD. The data was analyzed using JMP ver.16.0.0.

Result: The study population (n = 467) over a period of 14 years had male preponderance (67.45%) with a median age of 24 years (0.125–62). An average of 2.4 ± 1.47 relatives were screened per patient with average matching being 0.26 ± 0.48. A total of 115 patients had full match with a mean age of 24.96 ± 13.19y, male preponderance (52%), and maximum were with siblings (50%-brothers,45%- sisters). Though 41% patients' parents were screened with only 3% mother, and 2.

Conclusions: This study highlights that in Indian scenario the chances of full match are 1 out of every 4 tests per family. Screening parents are futile despite in-family marriages in our country.

Differentiation of Aplastic Anemia from Myelodysplastic Syndrome Using Cell Population Data Analysis on Sysmex XN-9000 Automated Hematology Analyser

Sreya P B, Rutvi G Dave, Tulasi Geevar, Nitty Mathews, Jansi Rani, Sukesh C Nair

Introduction: Sysmex XN-9000 (Sysmex Corporation, Kobe, Japan) automated hematology analyser has new parameters—Cell Population Data (CPD) to assess morphological alterations of neutrophils, lymphocytes and monocytes.

Aims & Objectives: We aim to utilise novel parameters to objectively identify dysplasia in Myelodysplastic syndrome (MDS) and differentiate it from other cytopenia like Aplastic Anemia (AA).

Materials & Methods: Retrospective study consisting of 120 healthy controls, 51 cases of AA and 28 cases of MDS. Cases in which bone marrow examination was done were included in the study.

The WBC differential count was performed in the White Differential channel (WDF), each cell was classified and presented in the three axes of the WDF scattergram based on internal complexity and granularity (side scattered light, SSC: X-axis), RNA/DNA content (side fluorescence intensity, SFL: Y-axis) and size (forward scattered light, FSC: Z-axis). Median positions and width of dispersion of neutrophils (NE-SSC, NE-SFL, NE-FSC & NE-WX, NE-WY, NE-WZ), monocytes (MO-X, MO-Y, MO-Z) and lymphocytes (LY-X, LY-Y, LY-Z) on each axis of the scattergram were obtained. Other novel parameters (IPF,nRBC, FRC,RET-Y,RET-RBC-Y,IRF-Y) were also compared between the 2 groups.

Result: MDS cases had lower NE-SSC, NE-FSC compared to reference range whereas IG%, NE-WX, NE-WY, NE-WZ, IPF, nRBC, FRC were elevated.

AA cases had all the parameters comparable to reference range.

On comparing the two groups under WBC parameters NE-SSC, NE-SFL, MO-Z were significantly lower in cases of MDS while LY-X, NE-WX were significantly higher in MDS, which may be attributed to dysplastic changes in the cytoplasm and nucleus such as hypo granularity and abnormal segmentation.

RET-Y, RET-RBC Y, IRF-Y were significantly lower in cases of MDS and IPF, nRBC, FRC were significantly higher in MDS.

We established sensitivity, specificity of the above parameters. FRC and RET-Y had the best area under the curve (AUC) of 0.839 & 0.833 respectively.NE-SSC had AUC 0.812, sensitivity of 75% specificity of 84%; NE-SFL had AUC 0.815, sensitivity of 85% specificity of 76.5%. IPF had (AUC) 0.785, sensitivity of 66.67, specificity of 86.96%. This may be due to platelet anisocytosis due to dysmegakaryopoeisis.

Conclusions: CPD analysis showed that patients with MDS and AA showed significant changes in the complexity, content and size of neutrophils, monocytes and lymphocytes. This may aid in differentiating hypoplastic MDS from evolving aplasia which is a close differential diagnosis.

Items	MDS (n=28)	AA (n=51)	P Value*	Reference Range(n=120)
NE SSC	141.711 (14.0446)	153.067 (9.3545)	<0.001	151.81 (144.5-157.4)
NE SFL	49.768 (8.7206)	57.488 (6.3899)	<0.001	52.65 (47-58.2)
NE FSC	79.711 (8.996)	82.975 (10.9073)	0.233	88.78 (83-94.5)
LY-X	81.782 (2.0662)	79.792 (2.6948)	0.002	79.89 (77.1-82.6)
LY-Y	70.700 (10.68)	69.704 (5.3648)	0.509	73.755 (67.25-80.25)
LY-Z	57.846 (57.8)	58.076 (1.1167)	0.913	59.08 (57.54-60.62)
MO-X	117.761 (118.8)	117.708 (4.8387)	0.840	118.67 (120.4-116.2)
MO-Y	112.607 (116.96)	109.884 (22.5422)	0.513	119.76 (106.8-132.6)
MO-Z	63.368 (5.36)	64.614 (9.2420)	0.003	66.43 (63.2-69.6)
IG%	2.0214 (2.78313)	.7978 (1.66)	0.140	0.733
NE-WX	378.089 (104.99)	302.402 (90.37)	0.010	300.66 (274-326)
NE-WY	724.321 (168.09)	643.373 (177.23)	0.140	590.82 (538-642)
NE-WZ	698.857 (103.29)	604.000 (147.4296)	0.100	615.52 (553-677)
IPF	18.900 (15.2003)	6.543 (5.67)	<0.001	
nRBC	1.614 (4.2057)	0.361 (.47)	0.328	0.018
FRC	2.9064 (2.25178)	.6085 (.85)	<0.001	
RET-Y	161.936 (2.25178)	185.030 (13.12)	<0.001	
RET-RBC Y	161.732 (15.5236)	169.737 (6.41)	0.002	
IRF-Y	161.229 (32.34)	173.796 (32.33)	0.021	

ROC ANALYSIS RESULTS TO IDENTIFY BEST CUT OFF OF THE TEN CELL POPULATION DATA (CPD) ITEMS WITH SIGNIFICANT DIFFERENCES FOR DISCRIMINATION OF PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) FROM APLASTIC ANAEMIAS

ITEMS	AUC (95%CI)	BEST CUT OFF (95%CI)	SENSITIVITY (95%CI)	SPECIFICITY (95%CI)
NE SSC	0.812	≤150	75.00	84.31
NE SFL	0.815	≤54.6	85.71	76.47
LY-X	0.709	>80.4	75.00	58.82
MO-Z	0.664	≤64	53.57	74.51
NE-WX	0.772	>347	60.71	80.39
IPF	0.785	>9.5	66.67	86.96
FRC	0.839	>0.74	85.71	80.43
RET-Y	0.833	≤180.2	82.14	73.91
RET-RBC-Y	0.663	≤161.7	46.43	91.30
IRF-Y	0.714	≤172.9	75.00	67.39

Values represented as mean with Standard Deviation in brackets

*Statistical significance between AA and MDS

Abbreviations: LY-WX, width of dispersion of lymphocyte complexity; LY-WY, width of dispersion of lymphocyte fluorescence; LY-WZ, width of dispersion of lymphocyte size; LY-X, lymphocyte complexity; LY-Y, lymphocyte fluorescence; LY-Z, lymphocyte size; MO-WY, width of dispersion of monocyte fluorescence; MO-WZ, width of dispersion of monocyte size; MO-X, monocyte complexity; MO-Y, monocyte fluorescence; MO-Z, monocyte size; NE-FSC, neutrophil size; NESFL, neutrophil fluorescence intensity, NE-WY, width of dispersion of neutrophil fluorescence; NE-SSC, neutrophil complexity; NE-WX, width of dispersion of neutrophil complexity; NE-WZ, width of dispersion of neutrophil size; IPF- immature platelet fraction; FRC-Fragmented RBC; RET-Y- reticulocyte parameter; IRF-Y-immature reticulocyte fraction.

Sigma Metrics in Haematology: Are Second Party Controls a Better Alternative?

Khevna Kansara, Monica Gupta, Mustafa Ranapurwala, Mitul Chhatriwala

Introduction: While the Sigma concept is not new to quality. For analytical processes in the laboratory, Sigma metric analysis indicates

the level of quality control achieved and indicates how far a given process deviates from perfection.

Aims & Objectives: The study aimed to assess the application of sigma test in selected Hematology parameters a and compare the same while using third party controls (TPC) and second party controls (SPC).

OBJECTIVES

1. To calculate the sigma metrics for selected hematological parameters with SPC and TPC.
2. To use quality goal index (QGI)for unacceptable sigma levels (< 3 >
3. To plot an OPSpecs chart for all the parameters and assess the overall performance.

Materials & Methods: This observational study was carried at Central Diagnostic Laboratory of Shree Krishna Hospital, Karamsad, Gujarat. Sigma metrics was calculated for 5 batches of TPC and SPC each and compared.

Result: Average sigma metrics achieved with TPC for IQC and EQAS bias was RBC: 6,4.7; HB: 6.4, 4.2; HCT: 5.8, 3.3; PLT: 6.2, 4.7; and WBC: 2.1, 2.5 respectively. Average sigma metrics achieved with SPC for IQC and EQAS bias was RBC: 4.5,6.7; HB: 4.1,4.1; HCT: 4.3,4.2; PLT: 13.2,8.4 and WBC: 7.5,7.5 respectively. The laboratory has achieved excellent to world class performance in all analytes with SPC and TPC except WBC, with TPC showed average performance. QGI showed that the WBC had problem of inaccuracy with IQC and EQAS bias. OPSpecs chart were prepared to assess the overall performance.

Conclusions: Labs must preferentially use the sigma metrics and sigma QC rules to design its internal QC protocols, for individual parameters, rather than follow the 'one fit for all' recommendation. Normalized OPSpecs chart function as report cards for the lab and analyzer QC performance, along with suggestions for applicable rejection rules. Although TPC is generally preferred because they provide an unbiased and independent assessment; for Hematology that relies on controls with short half-life; where labs are dependent on manufacturer's assigned mean and standard deviations, SPCs may be a better alternative.

Performance Evaluation of Digital Cell Imaging by Sysmex DI-60 integrated into automated hematology analyzer system

Debirupa De, Rajesh Kumar Bhola, Debahuti Mohapatra

Introduction: The manual microscopic evaluation of peripheral blood plays an important role in the diagnosis of hematological disorders. But it requires skilled morphologists but still have the subjective variation with high imprecision. Automated hematology-analyzers can reduce the turnaround time (TAT) with high precision but requires slide review for abnormal cases based on flagging rules. In this regard, automated digital morphological assessment may provide results with shorter TAT & high precision. The Sysmex XN series analyzers have been equipped with a modular system of automated CBC analysis followed by slide making & staining using reflex rules & reviewing the slide by digital scanning & pre-classification.

Aims & Objectives: The performance of automated digital image analyzer, Sysmex DI-60 was evaluated based on pre-classified differential counts & compared with manual differential counts on 200 cells using CLSI H20-A2 guidelines.

Materials & Methods: Total 7072 samples were processed by the Sysmex analyzer over one month to evaluate the processing ability of XN series analyzers connected with DI-60. Out of which total 450 samples were selected randomly using various flagging criteria from the automated CBC analyzer to compare with manual microscopy. The digital images were reviewed for correct reclassification.

Result: Out of 7072, total 49 (0.7%) samples failed to be analyzed by the digital scanning due to various technical reasons which required putting the slides for repeat scanning. The average time taken for scanning a slide was 2 min. The pre-classification of WBC was most accurate for segmented neutrophils followed by other cells. The pre-classification error commonly encountered was wrong classification of few monocytes or immature granulocytes as pro-myelocytes. But it has identified correctly promyelocytes in APML & blasts in leukopenic samples. The correlation coefficient of most of the parameters was > 0.8. The correlation was improved with post-classification of cells.

Conclusions: Although the performance of Sysmex DI-60 in automated pre-classification of various leukocytes is acceptable, still reviewing the digital images & post-classification for proper reassignment is necessary. But the introduction of a reflex system of slide making, staining & scanning by DI-60 reduced the TAT & increased the efficiency of the laboratory.

Whole Exome Mutation Study in Triple Negative Polycythemia: An Indian Quest

Harikrishnan Premdeep, Harshit Khurana, Anil K Madugundu, Babylakshmi Muthusamy, Shankar Subramanian, Sriram Kishore Garapati, Akhilesh Pandey, Uday Yanamandra

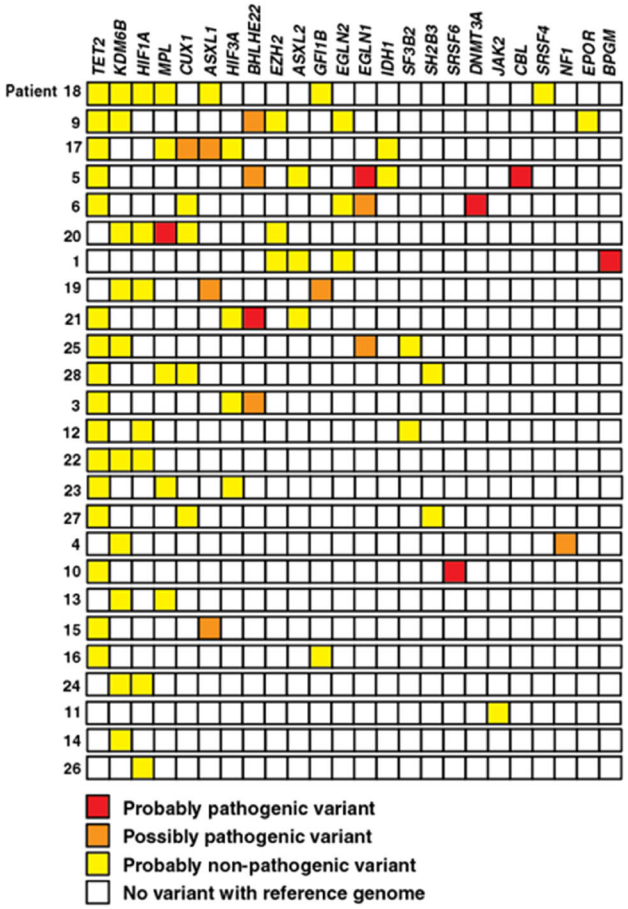
Introduction: Polycythemia encompasses a varied spectrum of disorders characterized by an increase in the red cell compartment in peripheral blood. It is classified as either primary or secondary erythrocytosis based on where the defect lies and subclassified as congenital or acquired. Polycythemia vera, the most common primary acquired erythrocytosis results from mutations involving the JAK2 gene in up to 98% of cases. Although numerous other mutations have been identified in the causation of polycythemia, in up to 80% of these cases, the causal genetic defect remains unidentified and there is an absolute dearth of reliable information on the incidence and prevalence of these mutations particularly in Indian patients.

Aims & Objectives: To evaluate the spectrum of genetic mutational defects in Indian patients with Triple negative polycythemia.

Materials & Methods: All cases of polycythemia that were triple negative (JAK2 mutations (Exon 12/14), cMPL, and CALR) or acquired secondary causes were subjected to genetic analysis for EPOR, VHL, PHD2, HIF2 alpha, alpha- and beta-globin gene mutations. After an analysis of the whole exome sequencing, bioinformatics analysis was performed to identify variants that may be involved in the pathology of polycythemia and thereby establish causal inferences.

Result: Of the total study population of 28 Triple-negative polycythemia, 25 patients had other mutations wherein 13 were "probable pathogenic" and six were "possible pathogenic" (Fig. 1). Among these mutations, most patients had variants of the gene involved in epigenetic processes like TET2 (n- 16; 64%) and ASXL1 (n-4; 16%), and hematopoietic signaling pathways like MPL (n- 06; 24%), and GFIB (n- 03; 12%). Based on bioinformatics analysis, the conservation status of nucleotides and amino acids in these variants were identified which would translate to the clinical implication of these variants.

Conclusions: All patients with Triple Negative Polycythemia can still have genetic mutations attributed pathologically involving epigenetic processes and hematopoietic signaling pathways.



Study of BCR-ABL1 Kinase Domain Mutation Profile: A 10 Years Experience of a Tertiary Cancer Care Center

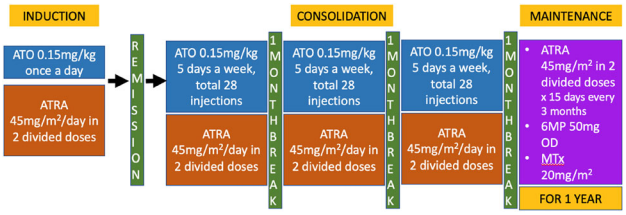
Debajani Nathi, Chumki Bhattacharjee, Poonam Das, Indranil Dey, Rakesh Demde, Arijit Nag, Jeevan Kumar, Saurabh Jayant Bhave, Reena Nair, Asish Rath, Mayur Parihar, Sushant S Vinarkar, Deepak Kumar Mishra

Introduction: Chronic myeloid leukaemia(CML) is a clonal haematopoietic stem cells disorder characterized by the presence of Philadelphia chromosome(Ph chromosome); t (9;22)(q34.1; q11.2). The Ph chromosome is associated with the production of BCR-ABL1 fused protein with high ABL tyrosine kinase activity and recognized as hallmark for pathogenesis of the disease. Imatinib is used as a frontline therapy for CML and is a selective inhibitor of tyrosine kinase that binds to the ATP-binding site of BCR-ABL gene. Mutations in the ABL1 kinase domain of BCR-ABL1 fusion transcript is known as one of the important causes leading to imatinib resistance during CML management.
Aims & Objectives: Study the mutation profile of BCR-ABL1 kinase domain in CML.
Materials & Methods: We performed retrospective analysis of 219 samples referred for ABL1 kinase domain mutation analysis over last 10 years. Depending upon the transcript type, major breakpoint cluster region (M-BCR) and minor (m-BCR) breakpoint cluster region,we used a reverse transcriptase polymerase chain reaction followed by bidirectional sanger sequencing to document resistant mutation in the ABL kinase domain. The procedure involved a first-round amplification starting with the BCR-ABL1 fusion gene (from BCR exon 2 to ABL1 exon 10) followed by a 2nd nested PCR amplification across the TK domain (ABL1 exon 4–10).
Result: Among 219 samples, we found 47 cases with pathogenic missense mutation, 11 cases with deletions (7 cases—exon 6 del, 4 cases—exon 7 del) and 154 cases with no pathogenic mutations in the ABL1 kinase domain. T315I (n = 17) mutation was a frequently observed variant followed by F317L variant in the Imatinib resistant cases. Compound mutations (> 2 mutations) were identified in the 7 cases, of which 4 cases had received multiple TKI therapies. Out of the 47 cases with pathogenic missense mutation in ABL1 kinase domain, change in therapy was documented in 26 cases which was followed by good response to therapy in 19 cases.
Conclusions: We documented frequency of 21.

Long-Term Real-World Outcomes of Patients with High-Risk Acute Promyelocytic Leukemia Treated with Arsenic Trioxide and All Trans Retinoic Acid Without Chemotherapy

Charanpreet Singh, Uday Yanamandra, Parathan Karunakaran, Nishant Jindal, Saloni Rani Kumari, Neha Saini, Aditya Jandial, Arihant Jain, Chandan Das, Deepesh Lad, Gaurav Prakash, Alka Khadwal, Shano Naseem, Reena Das, Neelam Varma,Subhash Varma, Pankaj Malhotra

Introduction: The treatment of Low and Intermediate risk Acute Promyelocytic Leukemia (APML) with Arsenic Trioxide (ATO) and All Trans Retinoic Acid (ATRA) has become the standard of care. However, most protocols include the addition of chemotherapy for high-risk patients.
Aims & Objectives: To study the efficacy of ATO and ATRA without chemotherapeutic agents for treatment of high-risk APML.
Materials & Methods: All patients diagnosed with APML with RTPCR positive for PML-RARA from 2006–2021 were included in the study and their records were reviewed. Patients received therapy as per protocol shown in Figure 1. Hydroxyurea was given for cytoreduction, including in supra-therapeutic doses on physician discretion. After a protocol modification in 2015, oral Prednisolone (0.5-1 mg/kg/day for 14 days followed by taper) was added to the induction therapy and maintenance therapy was reduced to 1 year. The per-protocol population refers to the patients who did not die within 7 days of initiation of therapy.
Result: Eighty-Nine patients with high-risk APML were included in the study. The median age was 31 years. At presentation, 80 (89.9%) had bleeding manifestations including 13 patients (14.6%) with a major bleed. Five patients (5.6%) had thrombosis at presentation. The median number of days to achieve CHR was 32 (Range 16–65). Forty-five patients (50.6%) had an infection during induction and Differentiation syndrome (DS) was seen in 27 patients (30.3%). One patient defaulted during induction, and 23 patients died during induction, including 18 patients who died within 7 days of therapy initiation. Of the 65 patients who completed induction, 61 were in remission and 52 patients (80%) achieved molecular remission. The median follow-up for the per-protocol population was 42 months(Range 0–164). Two patients relapsed during follow up- 1 during maintenance and 1 after completion of therapy. Three patients died post-induction during follow-up. Median EFS and OS were not reached for the study population. The 5-year EFS and OS in the per-protocol population were 87 ± 7.8% and 88 ± 7.8%, respectively. The 5-year EFS and OS in the entire cohort were 69 ± 9.8% and 70 ± 9.8%,respectively.
Conclusions: Treatment of all high-risk APML with ATO and ATRA without chemotherapy is associated with excellent long-term outcomes in the real-world setting.



Feasibility and Safety of Using Gemtuzumab Ozogamicin in the Treatment of Acute Myeloid Leukemia: Real World Data from a Tertiary Care Cancer Center in India

Vinay Anand Guntiboina, Vivek Radhakrishnan, Arijit Nag, Jeevan Kumar, Saurabh Bhave, Deepak Mishra, Reena Nair, Mammen Chandy

Introduction: Acute myeloid leukemia (AML) is the most common acute leukemia in adults. Intensive chemotherapy and stem cell transplantation (HSCT) was the only curative modality till recently. GemtuzumabOzogamicin (GO) is a monoclonal antibody drug conjugate targeted against CD33 approved for the treatment of newly diagnosed AML.
Aims & Objectives: The primary objective of this study was to assess the safety and feasibility of using GO in the treatment of AML. Survival outcomes, including overall and event free survival, are secondary objectives of the study.
Materials & Methods: This is a single center, observational study of patients diagnosed with AML having received GO. Data was collected between November 2011 & 2021. Two subsets of patient populations received GO: relapsed/refractory and upfront therapy of newly diagnosed cases of AML. Descriptive statistics were analysed in Microsoft Excel. Categorical variables and survival outcomes were analysed with SPSS version 26.0.
Result: Seventeen patients were analyzed in this study, of which 11 were newly diagnosed. A total of 39 infusions were administered. A third of the patients (35.3%) were older than 50 years. Forty seven percent (47%) of the patients had intermediate risk AML. Patients in the relapsed/refractory cohort were younger, with a median age of 14. The most common non-hematological adverse event (AE) was ≥ grade III transaminitis (25.6%) followed by gastrointestinal AEs (15.3%) (Table 1). Veno-occlusive disease (VOD) was observed in 2 patients. Infusion related reactions (≥ grade 2) were noted in 3 patients, accounting for 7.7% of all infusions. Sixteen episodes (41%) of febrile neutropenia were reported, with 10% having septic shock and 10% requiring ICU stay. At 2 years, median overall survival (OS) in the entire cohort of patients was 57%, with a 4 patients (23.5%) relapsing post therapy. Amongst the patients treated upfront with the addition of GO to intensive chemotherapy, including favorable and intermediate risk patients, median OS at 2 years was 70% (Figure 1).
Conclusions: We present the first real world data from India highlighting the use of GO in the therapy of AML, which has shown a toxicity profile similar to reported literature, suggesting that it is a safe addition to current intensive chemotherapy regimens. With use of compassionate access programs, it is feasible to provide wider accessibility to this immunotherapeutic modality.

Fig. 1: Kaplan Meier survival curves depicting overall survival (OS) data of patients treated with Gemtuzumab with a median 2 year OS of 70% in the patients treated with GO upfront (left) and 57% in the entire cohort (right)

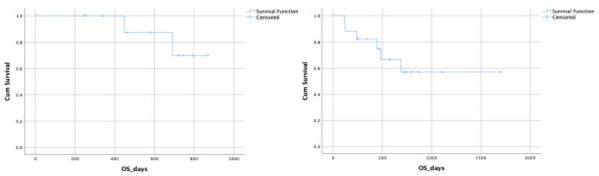


Table 1: Adverse event profile of the entire patient cohort. The total number of patients analyzed was 17 and a total of 39 infusions were administered.

Adverse event	n	Frequency (%)
Infusion related reaction	39	3 (7.7)
Haematological toxicity		
Neutropenia > 7 days	39	30 (76.9)
Thrombocytopenia > 7 days	39	38 (97.4)
Anaemia	39	16 (41.0)
Non haematological toxicity		
Transaminitis	39	10 (25.6)
Gastrointestinal	39	6 (15.3)
Neuropathy	17	1 (5.8)
Cardiotoxicity	17	0 (0)
Veno occlusive disease (VOD)	17	2 (11.8)
Infection		
Febrile neutropenia	39	16 (41.0)
Septic shock	39	4 (10.2)
ICU stay	39	4 (10.2)

Diagnostic Splenectomy: Clinical Profile, Role and Relevance in Diagnosis of Lymphoma

Nutan Joshi, Uday Kulkarni, Sushil Selvarajan, Kavitha M Lakshmi, Elanthenral S, Santhosh Raj, Abi Manesh, DivyaNinan, Junita John, Aby Abraham, Vikram Mathews, Anu Korula

Introduction: There is paucity of data regarding the role and relevance of a diagnostic splenectomy.
Aims & Objectives: To describe the clinical profile and outcomes of diagnostic splenectomies.
Materials & Methods: Total 1165 splenectomies were performed at our center from January 2008- June 2022. We analyzed the clinical profile and outcome of diagnostic splenectomies after excluding therapeutic and traumatic splenectomies.
Result: 123 diagnostic splenectomies were done in this period. 75 (61%) patients were male and median age was 44 (range 17- 74) years. 'B' symptoms were present in 82 (66.7%) patients. Lymphadenopathy (LNE) was noted clinically/on imaging in 29 (23.9%) patients, though lymph nodes were not amenable for biopsy or biopsy had failed. Median pre-surgical spleen size on imaging was 20 cm (range 11–37). 53 (43.1%) patients noted to have focal lesions within the spleen, with median max dimension of 9 (0.3–11.5) cm. Lymphocytosis (> 50%) was seen in 13 (10.6%) patients. Serum LDH was elevated in 66.7% of evaluated cases. Amongst 115 patients, for whom bone marrow report was available, 52 (45.2%) patients had normal bone marrow findings, 45 (39.1%) showed lymphoma or suspicious of lymphoma, infection in 5 (5.2%) patients and other diagnosis in 13 (11.3%). However, of 18 patients where immunophenotyping was also available subtyping lymphoma was possible in only 2 patients. (One splenic marginal zone lymphoma and one Hairy cell leukemia).
Splenic histopathology was diagnostic of lymphoma in 77 patients (62.1%), 5 (4.1%) showed suspicion of lymphoma, 12 (9.8%) revealed infectious causes, 2 specimens (1.8%) showed normal splenic morphology and 27 (22%) were non diagnostic with other findings. (congestion, extramedullary haematopoiesis, and lymphoid

hyperplasia). Table 1 describes the spectrum of subtypes of lymphoma cases.

Amongst all lymphomas, most common were B Non Hodgkin lymphomas in 48(62.3%) patients (Splenic Marginal Zone Lymphoma being most common, 26 [33.3%]) followed by T cell lymphomas in 17 (22.1%) patients (PTCL NOS being most common).

On univariate analysis, none of the clinical or laboratory parameter was found to be predictor of diagnosis of lymphoma.

Conclusions: Diagnostic splenectomy has utility in the diagnosis of lymphoma and specifically subtyping.

Unraveling the Somatic Mutational Landscape in Myelodysplastic Syndrome: A Single Center Experience

Indranil Dey, Sushant Vinarkar, Mayur Parihar, Kunjal Patel, Rakesh Demde, Kallol Saha, Saheli Banerjee, Debjani Nathi, Arijit Nag, Jeevan Kumar, Reena Nair, Mammen Chandy, Deepak Kumar Mishra

Introduction: Myelodysplastic syndromes (MDS) are clonal haematological disorders characterized by cytopenia, hematopoietic cell dysplasia and a predisposition to transform into Leukaemia. Tremendous interest in the complex genetics of MDS is evident by the emerging diagnostic, prognostic, therapeutic genetic biomarkers and as highlighted by the recent integration of mutation profile into the prognostic scoring of MDS (IPSS-M). High throughput next generation sequencing (NGS) has been instrumental in unravelling this complex genetic landscape of MDS.

Aims & Objectives: To study the genomic profile of patients with MDS using NGS at our center.

Materials & Methods: Routine workflow in assessment of MDS at our center involved peripheral blood, bone marrow examination, cytogenetic study and comprehensive myeloid panel (NGS). We studied the mutational profile of 46 cases of de-novo MDS diagnosed between March 2019 and May 2022. DNA/RNA was extracted from bone marrow samples, followed by library preparation using Oncomine Myeloid Research Assay. DNA and RNA sequencing was performed on Ion Torrent PGM platform. Sequencing reads were aligned using Torrent suite (5.12.1), followed by annotation of.BAM files with Ion Reporter (v 5.18.4.0).

Result: Over a 3 year period, 46 cases were diagnosed as MDS based on the clinical, morphological and cytogenetic findings out of which 57% patients were $\geq$ 60 years of age, M:F ratio was 1.8:1 and 79% had good cytogenetic risk (IPSS-R). 61% of cases had $\geq$ 1 pathogenic variant (n = 28/46). 39%(n = 11/28) of mutated cases had a single gene variant while rest had multiple (2–4) mutations. MDS-MLD (43%,n = 20/46) was the most common morphological classification and most frequent mutated sub-group followed by MDS-EB (30%,n = 14/46). According to functional genetic categories; majority of the mutated genes belonged to DNA methylation (50%,n = 14/28), followed by RNA splicing regulators(46%,n = 13/28) and transcription regulating genes (39%,n = 11/28). The most frequently mutated gene was *TET2* (39%,n = 11/28), followed by *RUNX1* (21%,n = 6/28) and most commonly co-mutated genes were *TET2* and *SRSF2* (29%,n = 5/17). 5 patients succumbed to disease/therapy related complications out of which 2 harboured mutations in *TP53* gene.

Conclusions: We studied the genetic profile of 46 MDS cases using NGS. The mutation frequency of MDS at our center was 61% which was concordant with published literature. *TET2* and *RUNX1* genes were the most frequently mutated genes and *TP53* gene mutated patients showed progression and poor outcomes.

Mono-Dysplasia Score Based on Automated Cell Counters: A Novel Parameter for Differentiating Reactive Monocytosis form Haematological Malignancies

Priyanka Moule, Sabina Langer, Nitin Gupta, Jasjit Singh, Jyoti Kotwal

Introduction: In India, there is a high burden of infections like tuberculosis, acute viral infections like dengue and malaria which are common causes of monocytosis. This increases the workload of smear examination to identify haematological malignancies that requires analysis by highly trained and experienced Pathologist. In contrast "Mono- dysplasia score" is obtained with a simple CBC on an automated cell counter, is operator independent, objective and doesn't require high level of expertise. We studies the utility of Monoscore to differentiate haematological malignancies form reactive monocytosis.

Aims & Objectives: To assess the utility of Monoscore calculated using research parameters of Sysmex XN automated cell counter, as a screening tool for differentiating reactive monocytosis from hematologicalmalignancies associated with monocytosis and To compare the lab specific monoscore value with the established cut off of monoscore for differentiating reactive monocytosis from hematological malignancies.

Materials & Methods: Samples sent in an EDTA vacutainer for routine investigation i.e. hemogram fulling the criteria for monocytosis (WHO criteria—absolute monocyte count $\geq 1 \times 109$/L and accounting for $\geq$ 10% of the total WBC) were included in the study. Monoscore calculated using the following formula:

1/(1 + exponential (-(-11.623 + 0.026 $\times$ Ne-WX -1.385 $\times$ Ne/Mo + 2.714 xMo value))).

PBS, flowcytometry and bone marrow examination done as and when needed as standard of care tests.

Result: 1257 samples analysed out of which 41 samples were confirmed chronic myelomonocytic leukemia and 126 were other hematologicalmaligancies including acute myeloid leukemia, chronic myeloid leukaemia, B cell lymphoblastic leukemia, myelodysplastic syndrome and other myeloproliferative neoplasm. A cut of 0.212 showed a sensitivity of 97.6% and specificity of 96.4% to differentiated reactive monocytosis form hematological malignancies while a cut off of 0.267 showed 100% sensitivity and 79.2%–96.9% specificity for differentiating CMML from other hematological malignancies and reactive causes of monocytosis.

Conclusions: Mono-dysplasia score with above mentioned cut off can be used to screen monocytosis. A sample showing monocytosis with a score less than 0.212 can be safely released without PBS examination, reducing the burden of slides to be reviewed especially in high throughput labs.

Clinicopathological and Immunophenotypic Characteristics of Hairy Cell Leukemia: An Institutional Experience

Somanath Padhi, Gaurav Chhabra, Prabodha K Das, Chinmayee Panigrahi, Prapti Acharya, S Karthick Velavan, Asutosh Panigrahi, K Vamsi Krishna

Introduction: Hairy cell leukemia (HCL) is a rare, indolent chronic lymphoproliferative neoplasm characterized by characteristic cellular morphology, distinct immunophenotypic features, and hallmark BRAFV600E muation that derives leukemogenesis and influencing disease biology.

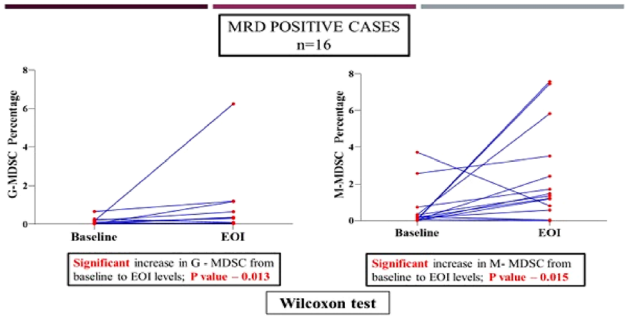
Aims & Objectives: To describe a case series of nine consecutive cases of HCL at our institution (2016–2022) and present three such cases with unusual presentation and a review of literature.

Materials & Methods: We describe the clinical features, laboratory hematological findings, bone marrow (BM) histomorphology, and immunophenotypic characteristics of nine cases of HCL (8 males, 1 female, median age; 54 years, range; 40–70 years) diagnosed over a period of six years and highlight three such cases with atypical presentation that created diagnostic dilemma.
Result: All *except* three presented with pancytopenia in the absence of clinically palpable spleen, liver, or lymphadenopathy mimicking 'aplastic anemia'; and showed very occasional atypical lymphomonocytoid cells morphologically intermediate between classical hairy cells (HCs) and prolymphocyte. Remainder six cases had circulating classical HCs, monocytopenia, with three showing lymphocytosis. The marrow aspirate (hemodiluted in six, dry tap in one) showed neoplastic lymphoid cells in all. Flowcytometry immunophenotypic analysis using peripheral blood and/or BM aspirate sample (n = 4/9) showed a low grade chronic lymphoprloliferative neoplasm with co expression of CD 11c, CD 103, CD 123, and CD 25 in seven (Matute score = 4/4), CD 26 (heterogeneous) in one, and aberrant co-expression of CD 23 in one. Trephine biopsy histomorphology revealed diffuse lymphoid infiltrate with characteristic '*fried egg*' like appearance, extensive reticulin fibrosis (MF grade 2 to 3), Annexin A1 immunopositivity in all but one (so called HCL-variant), and weak aberrant co-expression of CD 5 in two (cyclin D1 negative), and cyclin D1 (CD 5 negative) in two, and CD 10 in one. Mutant BRAFV600E testing was positive in two of the six cases (both cyclin D1 positive) where this was performed on paraffin block using RT-PCR technique. One out of nine cases had an unfavourable outcome where as remainder are currently under follow up.
Conclusions: Hairy cell leukemia in the absence of splenomegaly mimicking aplastic anemia is not uncommon; and this in presence of atypical immunophenotypic features may pose great diagnostic challenge. Awareness of such atypical presentation in classical HCL needs to be kept in mind and this require correlation with molecular testing for accurate diagnosis and appropriate management.

Myeloid Derived Suppressor Cells: A Silent Foe in Precursor B Cell Acute Lymphoblastic Leukemia

Mousumi Kar, Jasmita Dass, Ganesh Kumar V, Mukul Agarwal, Rishi Dhawan, Pradeep Kumar, Seema Tyagi, Tulika Seth, Manoranjan Mahapatra

Introduction: Myeloid derived suppressor cells (MDSCs) are circulating immune suppressive cells having a key role in immune suppression in cancer, tumor angiogenesis, drug resistance, tumor metastases. With the advent of newer immunotherapy agents targeting MDSCs, the Interest in these cells has skyrocketed in recent era. There are two predominant subsets: Granulocytic MDSC (G-MDSC), Monocytic MDSC (M-MDSC). Though its presence is a recognised fact in solid neoplasm, in leukemia it is still a grey zone.
Aims & Objectives: Characterization and quantification of MDSCs in the peripheral blood(PB) of precursor B-ALL patients at the time of diagnosis and post induction and correlate the results with post induction MRD level.
Materials & Methods: B-ALL patients taking treatment from the department of Hematology, AIIMS have been recruited from March 2021- July 2022. A 7-colour, 9-antibody panel has been used to analyse the PB samples. Acquisition done in flow cytometer- BD FACS CANTO-II. For analysis FACS DIVA software has been used.
Result: Fifty B-ALL cases have been recruited who have successfully completed induction. Post induction MRD was negative in 31 cases, whereas 16 cases were MRD positive and 3 cases were not in morphological remission. Both G-MDSC (median ~ 0.065%) and M-MDSC(median ~ 0.147%) levels were found to be higher in B-ALL cases at baseline than control samples. Both the levels are also higher in BMRD positive cases (G-MDSC median: 0.081% M-MDSC median ~ 0.32%) than BMRD negative cases (G-MDSC median: 0.03% M-MDSC median ~ 0.1%) in the baseline samples. It has also been observed that there is no significant increase in MDSCs from baseline to MRD time point in MRD negative cases; however, in BMRD positive cases these levels are showing a significant increasing trend (Figure 1).
Conclusions: Circulating immune suppressive cells (G-MDSC & M-MDSC) are significantly higher in B-ALL patients than normal control samples, more so in MRD positive cases. This study proves a possible role of cancer immunosuppression in B-ALL outcome and paves a way for newer cancer immunotherapy targeting MDSCs in leukemias.



Study of Association Between CRLF2 Overexpression and Post Induction MRD Status by Flow Cytometric Evaluation in Ph-Like B-All Patients

Vineeta Yadav, Prasanth Ganesan, Rakhee Kar, R Priyadarshini, M Prabhu, Raveendranath Veeramani

Introduction: In recent genomic analysis one subtype of B-ALL has been identified which shows similar gene expression profile with Ph-positive ALL with frequent IKZF-1 deletion but lacks the BCR-ABL1 fusion, known as Ph-like ALL. It is often associated with adverse clinical features, inferior outcome and high risk of relapse. It occurs in 10% in children, 21% in adolescent and 27% in young adult with B-ALL. Rearrangement of CRLF2 is the most frequent genetic alteration in Ph-like ALL associated with adverse outcomes. Minimal residual disease (MRD) study at the end of induction provides early information about prognosis of the patients.
Aims & Objectives: To study the correlation between CRLF2 expression and their post induction minimal residual disease status in B-ALL patients.
Materials & Methods: From June 2019 to May 2022, 122 newly diagnosed acute leukaemia cases of all age group were recruited. Fresh bone marrow sample were collected and processed by Flow Cytometry (FCM). The diagnosis of B-ALL was made by immunophenotyping. CRLF2 expression at the time of diagnosis and post induction minimal residual disease (MRD) were studied.
Result: The mean and standard deviation of age of paediatric group was 5.17 ± 3.33 years, for adults group was 55.29 ± 9.07 years and for adolescent and young adults (AYA) group was 22.63 ± 8.09 years. The overexpression of CRLF2 in paediatric group was 13.7%, in adults was 11.11% and in AYA was 17.74%. The survival outcome in paediatric group was CR—90.19%, median OS—8.77 months and median EFS—7.74 months. In adults, CR—44.44%, median OS—0.94 months and median EFS—5.74 months was observed. In AYA, CR—66.13%, median OS—8.14 months and median EFS—7.17 months was observed.

Conclusions: In this study we have analysed correlation between CRLF2 expression and their post induction MRD status. Based on this correlation, survival outcome of all age groups were in conformity with previous studies.

Poster Abstracts

Acute Leukemia-Clinical

Twice Daily vs Continuous Infusion of Cytarabine in Induction Chemotherapy of Acute Myeloid Leukemic Patients: A Retrospective Analysis from a Tertiary Care Centre

Ramesh Balasubramanian, Vandana Arya, Jyoti Kotwal, Nitin Gupta

Introduction: The standard treatment for fit AML patients is 7 + 3 induction therapy with Cytarabine 150-200 mg/m2 as 7 days continuous infusion and Daunorubicin 60-90 mg/m2 from days 1–3. The continuous infusion of cytarabine is very effective but has been associated with prolonged neutropenia of 2–3 weeks, sepsis and induction mortality of 10–15%. There is dearth of data regarding the alternate dosing schedule as opposed to continuous infusion of cytarabine. Twice daily infusion of cytarabine is easy to administer and does not require balloon infusion pump or dosifuser is an alternative dosing option to consider. But, there is lack of published data regarding the safety and efficacy of this approach.
Aims & Objectives: We did a pilot study to compare Continuous infusion with twice daily infusion of cytarabine for 7 days along with Daunorubicin for 3 days.
Materials & Methods: All adult AML patients of age > 18 years presented to our hospital in past 4 years with AML diagnosis having fitness for induction therapy were included in our study. Patients were classified according to ELN 2017 risk categories. NGS was done only in selected patients due to financial constraints. Comparison of Day 28 BM remission, refractory, infection and induction mortality rates amongst patients who received cytarabine 150 mg/m2/day for 3 h infusion twice daily for 7 days with patients who received continuous cytarabine infusion of 150 mg/m2/day for 7 days (Historical cohort) was made.
Result: The characteristics of 51 fit AML patients treated by Continuous Infusion (CI) Vs twice daily (BD) (Table 1).
The remission rate, refractory, death, bacteraemia and fungal pneumonia were not statistically significant between patients treated by Continuous Infusion Vs twice daily.
Conclusions: The twice daily infusion of cytarabine was not inferior to continuous infusion and has similar toxicity profile. Due to ease of administration, the twice daily infusion can be used in Induction therapy of AML. There is need for randomised control trial with large number of patients to establish the safety and efficacy of this dosing approach.

Table 1: Patient Characteristics with *ELN 2017 Risk stratification:

	Age (median = years)	Male (n=)	Female (n=)	Favourable* N= (%)	Intermediate* N= (%)	Adverse* N= (%)
CI (n= 28)	44	18	10	11 (39.3)	13 (46.4)	4 (14.3)
BD (n= 26)	52	14	12	12 (46.2)	9 (34.6)	5 (19.2)

Clinical Profile, Treatment and Outcome of Pediatric "Coffee Bean" Histiocytosis: A Single Center Experience

Pankhudi Priya, Faheema Hasan

Introduction: Langerhans Cell Histiocytosis is a group of diseases with a myriad of clinical manifestations and biological behavior, characterized by proliferation and accumulation of Langerhans cells in different organs.
Aims & Objectives: This study is to demonstrate the varied clinical presentations and the treatment outcome of the children in one of the few pediatriccenters in North India that cater to various benign and malignant blood disorders of children.
Materials & Methods: The study describes the 4-year experience of our center in managing children with LCH. The clinical presentations, relevant laboratory, and radiological findings, treatment, and outcome of all the 14 children presenting between December 2018 and August 2022 were retrieved from our Hospital Information system. The LCH IV protocol was taken as the backbone for classification, risk organ involvement, stratum allocation, for deciding on treatment strategy, treatment response, and outcome.
Result: A total of 14 children were diagnosed with LCH during the study period. The mean age at diagnosis was 36 months(range: 10–110 months, median: 34 months). The male: female ratio was 9:5. 12 children had multisystem LCH while 2 children had single system LCH. Lymphadenopathy was the most common clinical presentation while the skeletal system was the most common system involved, affecting 64% of the children. There were a total of 8(57%) children who were in complete remission, of which 1 child expired and one relapsed, though went into the second remission. One child had a progressive disease. Three(21%) children left against medical advice while 1 child abandoned treatment 2 weeks into therapy. One of the children became covid positive after diagnosis and expired before the treatment could be initiated.
Conclusions: The wide array of clinical manifestations warrants the need for a high index of suspicion for an earlier diagnosis of LCH. The morphological identification of Langerhan's cells and positive IHC for CD1a, S100, and/or CD207 are necessary for definitive diagnosis. All patients with LCH should undergo BRAF-V600E mutational testing to aid in diagnosis and treatment.

Prevalence of TPMT and NUDT15 Gene Polymorphism in Patients of Acute Lymphoblastic Leukemia Presenting With 6-Mercaptopurine Associated Toxicities-A Retrospective Analysis

Nivedita Prabhakar Yerramilli, Faheema Hasan, Aashi Gupta, Rajesh Kashyap

Introduction: 6-Mercaptopurine plays an important role in both induction and maintenance therapy of acute lymphoblastic leukaemia (ALL). The metabolism of this drug involves the enzymes thiopurine S-methyltransferase (TPMT) and nudix hydrolase 15 (NUDT15). It is ideally recommended to test all patients for gene polymorphism pre-emptively in order to prevent the associated toxicities.
In our centre, where in-house testing of enzyme mutation is currently not available and patient affordability is a matter of concern, certain pre-defined criteria are used, as per institution protocol, to choose the patients in whom testing is absolutely mandated.
Aims & Objectives: To determine the prevalence of TPMT and NUDT15 gene polymorphism among patients presenting with 6-MP related toxicity, and to assess the usefulness of pre-defined criteria in predicting the prevalence of enzyme mutation.
Materials & Methods: This study is a retrospective analysis conducted in Department of Hematology, Sanjay Gandhi Post-Graduate Institute, Lucknow. All patients that satisfy certain pre-defined cut-offs for haematological toxicity and hepatotoxicity after introducing 6MP were tested for enzyme polymorphism. 2 ml of peripheral blood was sent in EDTA vacutainers for targeted gene sequencing of common alleles of the enzymes. Data was collected retrospectively between September 2021 and August 2022 for analysis.

Result: During the study period a total of 78 patients underwent ALL induction out of which 20 had haematological toxicity and/or hepatotoxicity. 30% of them were diagnosed to have NUDT15 enzyme mutation and 33.33% were poor enzyme metabolizers.
35% patients had febrile neutropenia out of which 6 (85.7%) patients had NUDT15 mutation. The prevalence of enzyme mutation was significantly higher in those with febrile neutropenia. The prevalence of NUDT15 gene mutation was higher among those with acute neutrophil count $\leq$ 500/cumm and platelet count $< 30 >$
Conclusions: In centres where-in testing all patients pre-emptively is not feasible, pre-set cut offs for haematological toxicity and hepatotoxicity may be used after introducing the drug, to predict the prevalence of gene polymorphism.

Large Granular Lymphocyte Leukemia (LGLL): A Case Series Of Six Cases

Chetan Agarwal, Deepika Gupta, Amrita Saraf, Sabina Langer, Richa Chauhan, Jyoti Kotwal, Nitin Gupta

Introduction: LGLL are rare group of diseases with considerable difficulties in their correct diagnostic workup and therapy. The major challenges lie in their distinction from reactive lymphoproliferation (including autoimmune). There is marked heterogeneity in clinical presentation often including cytopenias or classical autoimmune phenomena further reflecting on treatment ranging from mere supportive measures over strategies of immunosuppression till chemotherapy. Here, we report a series of 6 selected LGLL cases from our tertiary care centre.
Aims & Objectives: To highlight variation in clinical presentation, immunophenotypic features and treatment outcomes in patients of LGLL.
Materials & Methods: A retrospective analysis of LGLL cases, diagnosed in our hospital, a tertiary care center in India from 2019 to 2022.
Result: These 6 patients include 5 male and one female with median age 45 years. All the patients presented with severe anaemia and only 2 with thrombocytopenia. All 5 males diagnosed as T-LGLL. The female patient diagnosed as NK-LGLL, had transfusion dependent anaemia with hepato-splenomegaly lost to follow up. All were treated with steroids, immunosuppressants and cyclophosphamide. Out of 5 cases diagnosed as T-LGL one died of pneumonia, one relapsed and three achieved hematological remission.
Conclusions: As this entity LGLL represents a diagnostic and therapeutic challenge. Our study represents spectrum of clinical manifestations, diagnostic features, management and outcomes of LGLL patients at our centre. Many patients showed indolent course requiring immunosuppressive therapy, however, there is need to highlight this heterogeneous nature of the disease required for correct diagnosis and management.

Clinical, Immunophenotypic and Cytogenetic Profile of Children With Acute Lymphoblastic Leukemia at a Tertiary Care Center in North India

Alka Yadav, Renu, Nikhil

Introduction: Diagnosis and risk stratification in childhood acute lymphoblastic leukemia (ALL) depends on the clinical, immunophenotypic, and cytogenetic profile of these patients at the time of diagnosis.
Aims & Objectives: Aims & Objectives: The study was carried out with an aim to look into the clinical, immunophenotypic, and cytogenetic profile of newly diagnosed children with ALL visiting a tertiary care hospital in North India.
Materials & Methods: Materials & Methods: This was the retrospective cross-sectional study to review the medical records of all children aged 1–14 years admitted to the pediatric ward between June 2017 to 31st May 2021 with a confirmed diagnosis of ALL. Clinical parameters, immunophenotypic, and cytogenetic profiles of these children were reviewed and analyzed.
Result: Results: A total of 127 patients were included in this study. The median age of the study population was 6 years (range 1 - 14 years) with a male (89): female (38) ratio of 2.4:1 in the study population. The most common presenting feature was fever (74.1%), followed by pallor (59.0%), bleeding manifestations (32.3%), and bony pains(22.8%). Some children also present with acute complications such as tumor lysis syndrome (9.4%), renal failure (7.1%), sepsis (6.3%), thrombosis (2.4%), and neurological involvement (3.9%) at the time of diagnosis. Hepatosplenomegaly at presentation was seen in 73.2% (93) of the study population followed by generalized lymphadenopathy in 40.2% of patients. Immunophenotypically, there were 86 cases (67.7%) of B-ALL and 39 cases (30.7%) of T-cell lineage. Of these, 92 (72.4%) cases expressed CALLA positivity (CD10). The subtype distribution of cases was as follows: pre B-ALL (66 cases; 51.9%), pro B-ALL (20-cases;15.7%); T-ALL (39 cases;30.7%). Aberrant expression was noted in 21 (16.5%) cases and was commonly seen in T-ALL. Aberrant markers noted in T-ALL were cCD79a and CD19. Cytogenetic study results were available for 115 out of 127 patients. Out 115, 12 were Philadelphia chromosome t(9:22), 11 had t(4:11), 6 had t(12:21) and 2 had t(1:19) detected multiplex RT-PCR and gel electrophoresis method. Based on clinical and cytogenetic profiles at diagnosis, 42 (33.1%) patients were high risk while 24 (18.8%) had intermediate risk, and the remaining 61 (48.1%) had the standard-risk disease.
Conclusions: Conclusion: Clinical assessment and cytogenetic-based risk stratification is crucial in childhood ALL management. With improvement in supportive care and the availability of targeted agents, the outcome of ALL continues to improve over time.

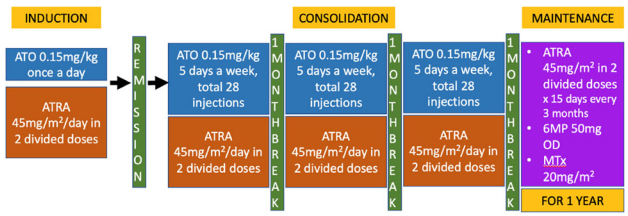
Use of a Supra-Therapeutic Hydroxyurea Dose as Cytoreductive Therapy in Patients with High-Risk APMLTreated with ATO + ATRA Without Chemotherapy

Sarthak Wadhera, Charanpreet Singh, Uday Yanamandra, Parathan Karunakaran, Nishant Jindal, Saloni Rani Kumari, Neha Saini, Aditya Jandial, Arihant Jain, Chandan Das, Gaurav Prakash, Alka Khadwal, Shano Naseem, Reena Das, Neelam varma, Subhash Varma, Pankaj Malhotra, Deepak B

Introduction: Induction therapy for High-Risk Acute Promyelocytic Leukemia (APML) usually includes a combination of Arsenic Trioxide (ATO) and All-trans Retinoic Acid (ATRA) with chemotherapeutic agents such as anthracyclines or Gemtuzumab. The use of supra-therapeutic doses of hydroxyurea as cytoreductive therapy in APML has not been studied.
Aims & Objectives: To study the efficacy of high-dose hydroxyurea during induction therapy in high risk APML.
Materials & Methods: We retrospectively analysed patients with high risk APML with a baseline TLC of $>$ 30,000/mm3 presenting to our center between 2016 and 2021. Patients were treated with ATO and ATRA without chemotherapy as per the protocol shown in Figure 1. For patients with high-risk APML, hydroxyurea was given as cytoreductive therapy, including in supra-therapeutic doses according to physician discretion. High-dose hydroxyurea dosing was defined as greater than 100 mg/kg/day with atleast a cumulative dose of 15 gm during hospital stay.
Result: Thirty-two patients had a TLC $>$ 30,000/mm3 at presentation during the study period, of whom, 15 (46.9%) received high dose

hydroxyurea during their induction therapy. The maximum hydroxyurea dose intensity ranged from 2gm every 6 h to a maximum of 2gm every 2 h. The Median cumulative hydroxyurea dose during induction was 57 gm (Range 8–112 gm). The Median number of days of hydroxyurea therapy during induction was 7 (Range 2–15 days). Patients who received high-dose hydroxyurea had lower early mortality- defined as death within 7 days of induction therapy- in comparison to patients who did not receive high-dose hydroxyurea (13.3% vs 52.9%; p-0.028). The median follow-up for the cohort was 22 months. On Kaplan–Meier analysis, patients who received high-dose hydroxyurea during induction therapy had a better overall survival in comparison to patients who received standard dose hydroxyurea (p-0.028).

Conclusions: Supra-therapeutic hydroxyurea is effective as cytoreductive therapy in patients with High-risk APML presenting with a very high leukocyte count.



ATRA Induced Hypercalcemia due to Azoles

Juhi Mehrotra, Santanu Sen

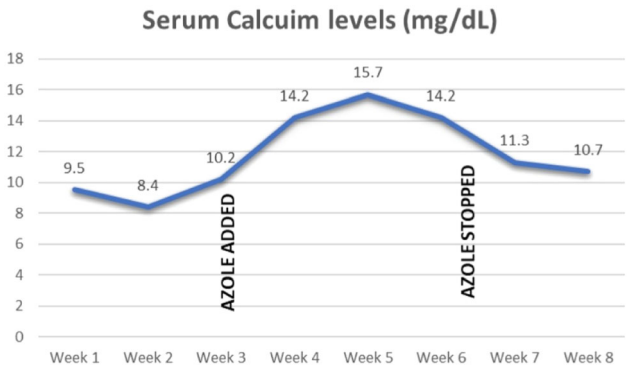
Introduction: ATRA has revolutionised the treatment of APML and has been the backbone of standard treatment protocols. Differentiation syndrome, pseudotumor cerebri and hyperlipidaemia are well documented side effects.

Aims & Objectives: Herein, we report a case of ATRA induced hypercalcemia in interaction with fluconazole in an APML case.

Materials & Methods: 11 year old boy on induction therapy during developed Differentiation syndrome and ATRA syndrome presenting with sudden onset headache, blurred vision and left lateral rectus palsy and responded well to steroids and discontinuation of ATRA. During consolidation, he received Fluconazole prophylaxis, and received ATRA at 45 mg/m2. He presented with severe backache and bone pains. Imaging was unremarkable but serum calcium levels were 14.2 mg/dL. Urine calcium: creatinine ratio was 1.34, vitamin D, Po4 and ALP and PTH were normal which precluded the possibility of primary hyperparathyroidism or ectopic PTH secretion.

Result: We withheld ATRA and treated with hyperhydration, diuresis and Pamidronic acid, and normalised in 1 week. 2 similar episodes noted on 5th day of ATRA in next 2 cycles at reduced dose of 25 mg/m2 with serum calcium 15.7 mg/dL, 14.2 mg/dL. Post intensive chemotherapy, fluconazole was stopped and he tolerated ATRA at 45 mg/m2 without hypercalcemia, thus surmising that hypercalcemia might have resulted as an interaction of ATRA with fluconazole.

Conclusions: ATRA metabolism involves liver cytochrome P450 subtypes 2C9 and 3A4 and azoles are inhibitors of these, causing increased plasma concentration of ATRA and increasing the effect on calcium metabolism. ATRA leads to enhanced osteoclastic activity with bone mineral resorption just like PTH and downregulates IL-6 receptors. ATRA induced hypercalcemia is rare suggesting possible interaction with azoles as a cause. We must watch out for this complication as it was not dose dependant, and did not present since the first dose of ATRA.



A Rare Case of Chronic Lymphocytic Leukemia Mimicking as Carcinoma Lung

Arijit Ghosh Mazumder, Priyanka Samal, Samir Sahu, Subhash C Dash

Introduction: Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder with clonal proliferation of functionally incompetent mature B lymphocytes, defined by an absolute lymphocyte count $> 5 \times 10^9$/ml malignant cells in the blood. The disorder is more common in men. In most of the cases, it is asymptomatic and diagnosed during routine blood investigations while getting evaluated for some other disease. However, patients need treatment once they are symptomatic with painless enlarged, bulky lymphnodes, anemia or thrombocytopenia.

Case report: We report a case of 67 year female, with Type-2 DM and HTN, who presented with difficulty in breathing to the emergency during the first wave of Covid -19 pandemic. Chest X-ray revealed left mid zone opacity suggestive of a lung mass. CT Thorax done showed multiple mildly enlarged mediastinal lymph nodes, with lobulated heterogeneously enhancing mass in the prevascular compartment of anterior mediastinum compressing left upper lobar bronchus. USG guided core needle biopsy of left upper lobe mass was done and pleural fluid was analysed, which was suggestive of a lymphoproliferative neoplasm. Then CBC was advised which demonstrated lymphocytosis. Flow cytometry and IHC confirmed the diagnosis mature B-cell neoplasm Chronic Lymphocytic Leukemia (CLL).

Discussion: Pulmonary manifestation of CLL include, hilar and mediastinal lymphadenopathy. Lung masses are a rare presentation. Richter's transformation is a unique complication of CLL. However, patients with CLL also have an increased risk of secondary malignancy most commonly Kaposi sarcoma, malignant melanoma and carcinoma lung. Our case is unique,as there were no features of Richter transformation that is weight loss, fever, night sweats, muscle wasting and increased hepatosplenomegaly or lymphadenopathy. She was evaluated for respiratory distress and diagnosed on Lung mass biopsy as atypical lymphoid cell infiltration and not as lung carcinoma or transformation to high grade lymphoma.

Conclusion: Clinicians should be aware that though chronic lymphocytic leukemia is diagnosed incidentally on routine blood investigations, atypical presentation must be kept in mind. A simple CBC and a peripheral smear examination gives a clue to the diagnosis at the earliest.

Cytogenetic Characterization of Mixed Phenotype Acute Leukaemia (MPAL): Experience from a Tertiary Care Centre

Manoj Moni V T, Shily Sipporah K, Subashni D, Arun Kumar Arunachalam, Sushil Selvarajan, Uday Prakash Kulkarni, Anu Korula, Biju George, Vikram Mathews, Poonkuzhali

Balasubramanian, Aby Abraham, Nancy Beryl Janet Arthur, Madhavi Maddali

Introduction: Mixed phenotype acute leukemia (MPAL) represents ~ 2–5% of acute leukemia cases. The blast cells of MPAL express multilineage immunophenotypic markers and may have a shared B/T/myeloid phenotype. Due to historical ambiguity in the diagnosis of MPAL, the genetics and clinical features of this disease remain poorly characterized.
Aims & Objectives: This study aimed to compare the roles of conventional karyotyping and FISH in the.
subcategorization of MPAL.
Materials & Methods: This retrospective study included all patients diagnosed as MPAL by immunophenotyping in the department of haematology, Christian Medical College, Vellore during 2018 to 2022. Review of patient's electronic records was performed to collect the clinical and laboratory characteristics at the time of diagnosis. Conventional karyotyping and FISH analysis was done according to standard protocols on all cases.
Result: The median age of the 19 patients (12 males and 7 females) included in this study was 24 years (Interquartile range: 14 to 33 years). Based on the karyotype, three patients were classified as MPAL with t(9;22)(q34.1;q11.2)/BCR::ABL1, MPAL with t(4;11)(q21;q23.1)/KMT2A rearranged in two patients, while three patients were reclassified as AML with recurrent cytogenetic abnormality—t(8;21)(q22;q22.1)/RUNX1::RUNX1T1. Among the remaining eleven patients (MPAL, B/myeloid, NOS in 1; MPAL, T/myeloid, NOS in 6 & MPAL, NOS in 4 patients), a complex karyotype was reported in three patients, isolated numerical and structural abnormalities in four and the remaining four patients had a normal karyotype.
Conclusions: Our study highlights the importance of cytogenetic analysis in the diagnosis and characterization of MPAL based on the revised WHO 2022 classification.

Cytogenetic characterization of acute megakaryoblastic leukaemia (AMKL): experience from a tertiary care centre

Introduction: Acute megakaryoblasticleukemia (AMKL) is a subtype of acute myeloid leukemia (AML) characterized by abnormal megakaryoblasts that express platelet-specific surface glycoprotein. It occurs in only 1% of adults with AML and 4–15% of children with AML. In AMKL, there are three subgroups: those with Downs syndrome (DS-AMKL), without Downs syndrome (non-DS-AMKL) and adults. Clinical and biological outcomes of DS-AMKL are superior to those of non-DS-AMKL.
Aim.

This study aimed to compare the roles of conventional karyotyping and FISH in the sub categorization of AMKL.

Patients and Methods.

This retrospective study included all patients diagnosed as AMKL by immunophenotyping in the department of haematology, Christian Medical College, Vellore during 2018 to 2022. Review of patient's electronic records was performed to collect the clinical and laboratory characteristics at the time of diagnosis. Conventional karyotyping and FISH analysis was done according to standard protocols on all cases. Molecular analysis was available for 2 patients.
Results: The median age of the 16 patients (12 males and 4 females) included in this study was 3.5 years (Interquartile range: 2 to 29 years). Among these, DS-AMKL was detected in 2 patients, one of whom had a RAM phenotype, and both had somatic *GATA1* mutation. The remaining non-DS-AMKL patients had heterogenous cytogenetic abnormalities that included t(1;22)(p13.3;q13.1)/*RBM15::MKL1* and t(9;22)(q34.1;q11.2)/*BCR::ABL1* in two patients each, *KMT2A* rearranged in three, complex karyotype in one patient, isolated numerical or structural abnormalities in 2 and normal karyotype in 4 patients. One among these patients had a synchronous mediastinal germ cell tumor. Based on the cytogenetic analysis, these patients of AMKL were re-classified as AML with recurrent cytogenetic abnormalities [t(9;22)(q34.1;q11.2), t(1;22)(p13.3;q13.1) and *KMT2A* rearranged AML], Downs associated AML and AMKL NOS.
Conclusion: The results of our study indicate that the non-DS-AMKL group has a heterogeneous spectrum of genetic abnormalities as well as the importance of cytogenetic analysis in the diagnosis and subcategorization of AMKL according to the revised WHO classification of 2022.

A Retrospective Study on Clinico-Biological Profile and Treatment Outcome of Mixed Phenotype Acute Leukemia Patients in a Tertiary Care Hospital

Manoranjan Swain, Sudha Sethy

Introduction: MPAL is a rare variety of acute leukemia diagnosed based on 2016 WHO classification of hematopoietic and lymphoid tumor characterised by blast cell of multiple lineage or blasts expressing markers specific to several lineages. Incidence of MPAL is 3–5% in adults and 2.4–3.7% in children. According to phenotypic expression MPAL shows biphenotype (B/T, B/Myeloid, T/Myeloid) or triphenotype (B/T/Myeloid).
Aims & Objectives: 1. To determine incidence of MPAL and its clinical correlation.
2. To determine incidence of the CHR achievement at the end of induction CT and over all survival
Materials & Methods: Retrospective study is being performed in all MPAL patients presented to HEMATOLOGY DEPARTMENT at SCB Medical College, Cuttack. All the data has been collected from the record section of SCB Medical College, Cuttack.
All leukemia patients were screened by leukemia panel. MPAL patients have been identified as per WHO classification criteria.

MPAL having dominant feature of myeloid leukemia had received 3 + 7 CT followed by 3 cycle of IDAC at standard doses. While dominant feature of ALL will receive BFI95 protocol. CHR will evaluated at the end of induction CT as per standard protocol.

Statistical analysis was SPSS software version V21.

Inclusion Criteria:

1. Both child and adult with MPAL are included.

2. Leukemia patients satisfying WHO classification criteria of MPAL.

Result: 1. MPAL is found in 34 cases, it is 3.5% of total cases, and fever is most common presenting symptoms.
2. Out of 34 cases of MPAL, 18 (52.9%) cases had B/Myeloid, 14 (41.1%) cases had T/Myeloid,and two cases had B/T/Myeloid.

3. Over all median survival of all the cases of MPAL was 10 months and median survival at the end of 15 month is 38%.
Conclusions: MPAL IS a rare acute leukemia in which accurate diagnosis is important for treatment because it has worse clinical outcome. Due to vast diversity in genotype, phenotype the disease is poorly understood, larger study are required for better understanding and search for better therapeutic approaches.

Study of the Role of Decitabine Maintenance in Acute Myeloid Leukemia (AML) in a Tertiary Care Teaching Hospital in West Bengal

Apurba Banerjee, Shuvam Bhattacharaya, Tuphan Kanti Dolai, Shuvraneel Baul, Sandeep Saha, Prokas Mondal, Rajib Dey, Shipla Roy, Abhishek Maurya

Introduction: Intensive induction, novel drug combinations with improved supportive care have led to higher response rates and improved survival for patients with AML. Consolidation therapy helps to eradicate residual leukemia. Despite these successes, relapse remains a major concern with relapse risk greater than 50% for all adults with the disease. Given the high rate of relapse, there is rationale and need for post remission maintenance therapy to mitigate this risk. Maintenance remains a standard of care for patients with acute lymphoblastic leukemia (ALL) but effective maintenance therapy for AML has not been established till date.
Aims & Objectives: To assess the efficacy of decitabine in AML maintenance.
Materials & Methods: This is a prospective study from January 2020 to August 2022. History taking & clinical examination were done of the patients admitted in hematology ward with a diagnosis of AML. Patients fit for intensive chemotherapy were given 3 + 7 induction. Based on post induction marrow remission status, consolidation therapy with three high dose cytarabine (HIDAC) were given. Post 3rd HIDAC, bone marrow aspiration was done to check for remission status. Allogenic or Autologous stem cell transplant were not considered due to logistic issues. Patients were put on Inj Decitabine @ 20 mg/m2/day I.V. for 5 days every 3 months and followed up by clinical examination, complete blood count, peripheral blood smear examination & bone marrow aspiration (BMA) every 3 month or earlier to check for disease remission status.
Result: Total 12(twelve) patients were followed up within this time period. Five patients are in remission for more than 12 months up to August 2022 from starting of Decitabine. Three patients are in remission for 6 months up to August 2022. Two patients experience relapse (16.66%). Median duration of remission is 10 months up to the follow up period.
Conclusions: Decitabine may be an effective maintenance therapy for AML patients. More study subjects & longer follow up period needed.

Incidence and Spectrum of Fungal Infections in Acute Myeloid Leukemia(Aml) Patients Receiving Induction Chemotherapy with Prophylactic Fluconazole: A Prospective Study In Odisha

Sonali Palai, Rabindra Kumar Jena, Sudha Sethy

Introduction: Invasive fungal infections (IFIs) are major cause of morbidity and mortality in AML with incidence of 12 to 34% in temperate countries with Aspergillus and Candida being the most common cause. However there is scant data regarding IFI epidemiology in tropical regions, where environments are suitable for fostering fungal growth. Posaconazole which is the antifungal prophylaxis of choice for AML patients, cannot be extensively used in a resource constrained setting due to its high cost. Therefore this study sought to determine the incidence and outcomes of breakthrough IFI in AML patients receiving induction chemotherapy with fluconazole and also compare its efficacy with posaconazole from historical data.
Aims & Objectives: Primary objective: Incidence of breakthrough IFI in AML patients undergoing treatment from Day 1 till bone marrow recovery.
Secondary objective.

- Outcomes in terms of 30 days mortality due to IFI in these patients after induction chemotherapy.
- Comparison of efficacy of oral fluconazole with that of oral posaconazole from historical data.

Materials & Methods: This was a prospective observational study conducted from April 2021-April 2022 in S.C.B MCH, Odisha with 106 confirmed cases of AML(other than AML-M3) in age group of 15–60 yrs. Patients with previous history of fungal infections, prolonged use of immunosuppressants were excluded. Participants received 3 + 7 chemotherapy with oral fluconazole at 3 mg/kg/day from Day 1 till bone marrow recovery. IFI was suspected if patient had fever refractory to 5 days of antibiotics, symptoms of respiratory tract infection, development of new infiltrations on chest X-ray or any other focal lesions like oral thrush, mass in nasopharynx. Blood samples were collected for microscopy on KOH mount and growth on sabouraud dextrose agar. HRCT thorax and bronchoalveolar lavage were done in suspected cases of pulmonary involvement. The incidence and spectrum of breakthrough IFI were noted. SPSS version25 was used for statistical analysis.
Result: Out of 106 AML patients, IFI occurred in 7 [Incidence = 6.6%]. Out of 7, 4 had invasive candidiasis[4/7 = 57%], 2 had invasive aspergillosis[2/7 = 28%] and 1 had mucormycosis[1/7 = 14%]. All patients of IFI could be salvaged except one with invasive pulmonary aspergillosis. 30 days mortality rate is 0.9%[1/106].
Conclusions: Incidence of breakthrough IFI in AML patients receiving induction chemotherapy with prophylactic fluconazole is 6.6% with mortality of 0.9%.Oral fluconazole is an effective and cheap prophylactic in preventing IFI and is non inferior to much expensive posaconazole. Resource constrained setting or overcrowded general ward didn't have any adverse outcome,neither on the incidence of IFI nor on mortality.

L- Carnitine Reverses L-Asparginase Mediated Hepatotoxicity in Patient with ALL: A Case Report

Bobby Abraham, Roshna P, Sruthy V, Elsa John, Reshma, Bonnie A G, Jesina S, Priya Prasad, Chepsy C Philip, Maria

Introduction: L Asparginase is a widely used antineoplastic agent, predominantly in the treatment of Acute lymphoblastic leukemia.derrived mainly From E coli/Erwinia chrysanthemi. This enzyme catalyzes the conversion of aspargine to aspartic acid and ammonia. Leukemic cells lack aspargine synthetase and thereby depend on serum levels of aspargine.Therefore depletion of aspargine can cause starvation of leukemic cells of aspargine which is required for synthesis of RNA, DNA and proteins leading to premature death of the malignant cells.
Aims & Objectives: L-Asparginase can cause significant hepatotoxic effects primarily due to to inhibition of hepatic protein synthesis.It can deplete the Clotting factors(II, V, VII, VIII, IX, prothrombin and fibrinogen) can cause transaminitis,increase in bilirubin levels and hepatic steatosis.
We report the potenital utility of L-carnitine and Vitamin B infusion in reversing L asparaginase hepatotoxicity.
Materials & Methods: A 46 yer old male was newly diagnosed to have ALL and was initiated on chemotherapy with BFM ALL protocol using Prednisolone, Vincristine, L-Asparginase, Daunorubicin and Methotrexate. There was minimal derangements in baseline liver functions even before the start of chemotherapy but here was a precipitous decline in the liver function after day 13 of chemotherapy. Serum bilirubin rose from 3.65 on day 13 to 9.55 mg/dl on day 19 of chemotherapy. There was a predominant increase in indirect bilirubin during the early stages associated with transaminitis and increase in alkaline phosphatase.
Result: Chemotherapy was stopped but bilirubin showed progressively increasing levels with predominant direct hyperbilirubinemia during the later stages. The peak levels of bilirubin was noted on day 24 of chemotherapy (Total bilirubin-24.46 mg/dl,Direct-15.37 mg/dl).We started him on treatment with L- Carnitine and Vitamin B infusion which resulted in dramatic recovery and normalisation of bilirubin levels.His bilirubin and liver enzymes normalised on day 30 after starting Chemotherapy and 7 days after starting treatment with L-carnitine and Vitamin B infusion.

Conclusions: This case report reiterates the pattern of seldom reported asparginase mediated hepatotoxicity which causes hepatic steratosis,cholestatic pattern of jaundice probably due to small duct injury/cholangiopathy and its successful resolution after treatment with L-carnitine and Vitamin B infusion.

Lumps and Bumps in Haematology: Leukaemia Cutis

Gloria Venissa Quadros, Safeera Abdul Khadar, Malcolm Pinto, Anuradha C.K. Rao, Indira Puthran, Rajesh Krishna

Introduction: Leukaemia cutis (LC) is a cutaneous disorder which is rare, referring to the infiltration of leukemic cells into the skin, with a prevalence of 2- 3% of leukemia patients. The infiltrated neoplastic cells can be myeloid or lymphoid in lineage The presentation can be in the form of papules, macules, plaques, ulcers and nodules.
Aims and Objectives: Acase report which showed the possibleassociation of leukemia cutis with the course of leukemic disease showing the blast count and flowcytometry results.
Materials and Methods: Clinical and laboratory data, histopathological diagnosis, bone marrow findings were retrieved from the hospital information system.
Result: A male patient aged 62 years presented with multiple erythematous nodules over trunk and extremities with the largest nodule measuring 6 × 6cms. A month old tender scrotal swelling was also present along with history of loss of weight and appetite with malaise since the last four months.
Investigations such as CBC showed pancytopenia with 10% blast cells on peripheral smear. Bone marrow showed 47% blasts suggestive of acute leukemia. Flowcytometry panel for acute leukemia suggested a diagnosis of CALLA-positive precursor B- ALL.

The biopsy of the skin lesion showed pan-dermal dense infiltration of atypical cells, subcutaneous tissue infiltrate and adnexal entrapment, which was suggestive of Leukemia Cutis which was then confirmed on immmunohistochemistry. The lesion responded well to induction and consolidation chemotherapy.
Conclusion: Leukaemia cutis is usually seen as a first clinical sign after the treatment suggestive of relapse. It is a diagnostic challenge on histopathology. The nodular skin lesion in our case guided us towards the diagnosis. A complete history, physical examination and an early skin biopsyhelps to arrive at a positive diagnosis to recognize this rare cutaneous manifestation of hematopoietic malignancies.

Four Cases of Chronic Myeloid Leukemia with Unusual Presentation: Divided by Phenotype United by Genes

Antonio D'Costa, Vivek Magar, Sai Vamshi Varanasi, Richa Juneja, Rahul Arora

Introduction: Hematologic malignancies are amongst the 10 common malignant disorders. Chronic myeloid leukemia (CML) is one of the common leukemia usually presenting in adults with splenomegaly and leukocytosis. Here we present series of 4 cases of CML with rather rare presentation creating mimicry to other hematological disorder.
Cases: We present another application of the genetic "power" available to us to uncover 4 cases with misleading clinical profile in past 6 months.
Case 1 is a 45 years old male, diagnosed as a case of Polycythemia Vera(PV) on bone marrow biopsy, however Jak2 was unmutated. He was on intermittent phlebotomies and aspirin for the past 4 years, but had recurrent coronary thrombosis. We did not believe the rare diagnosis of JAK2 Negative PV, and on further workup found him to be Chronic Myeloid Leukemia(CML) because of BCR-ABL1 p210 positivity.

Case 2 is a 55 year old female who was being evaluated for isolated thrombocytosis. Our clinical suspicion for isolated thrombocytosis without splenomegaly was essential thrombocythemia. Megakaryocyte morphology on bone marrow suggested the diagnosis of CML confirmed with BCR-ABL (p210) positivity.

Case 3 & 4 are 2 children who presented with priapism and splenomegaly. and leukocytosis(with left shift). Both had BCR-ABL p210 positive, and hence diagnosed as pediatric CML with unusal presentation of priapism. One was a known case of Sickle cell trait which possibly contributed to pathophysiology of priapism.

All 4 patients are started on imatinib and hematological remission achieved in 3 of them.
Discussion and Conclusion: Myeloproliferative neoplasm don't read text books. All these cases taught us that CML can potentially mimic ET and very rarely polycythemia vera. Pediatric CML is itself unusual and primary presentation being priapism is rather rare. Megakaryocyte morphology analysis and optimal use of genetic testing tools clinch the diagnosis. It is very important to get the correct diagnosis as the patient can be benefitted with tyrosine kinase therapy making his disease almost curable.

Eosinophilia Masquerading Acute Myeloid Leukaemia With t(8:21): A Rare Presentation

Sanjay Mishra, Mona V Khan, S P Verma, Anurag Singh, A K Tripathi

Introduction: Eosinophilia is routinely encountered in clinical practice and is mostly secondary to underlying pathology. Eosinophilia is associated with acute myeloid leukemia and various mutations like PDGFRA, PDGFRB AND FGFR1, BCR-ABL, inv(16) etc. and they are rare presentation. We encountered a case of eosinophilia masquerading acute myeloid leukemia with t(8:21).
Case: A sixteen-year-old boy arrived at the hospital with the chief symptoms of a 20-day fever, progressive abdominal enlargement, and generalised weakness with massive splenomegaly and hepatomegaly. There was no icterus, lymphadenopathy, or bleeding symptoms.
The hemogram revealed an 8.6 g/dl haemoglobin level and a total leukocyte count of 30 × 109/L, with enhanced eosinophils making up 72% of those cells. Eosinophils and their precursors (72%), promyelocytes (03%), neutrophils (12%), lymphocytes (06%) and blasts (07%) were detected in the peripheral blood smear (PBS) analysis. The platelet count was 20X109/L. Bone marrow examination too revealed 10% blast cells along with increase in eosinophilic precursors. Hence the diagnosis of Acute myeloid leukemia with eosinophilia was made.

Flow cytometry was performed on bone marrow aspirate. In CD45 versus side scatter, the blast cells were gated. There were 12% blasts and 70% eosinophils and their precursors. Bright positivity was seen for CD13 (64%), CD33 (65%), CD34 (38%), HLA-DR (62%), and MPO (42%) expression in the blast cells. B-cell and T-cell lymphoid markers were not present in the gated blasts. To validate this result and to rule out alternative eosinophilic leukemic disorders, cytogenetic investigations were requested. The results of a cytogenetic investigation using the conventional G-banding approach showed as 46, XY, t(8;21)(q22;q22) (Fig. 2). Cytogenetic testing was negative for PDGFR alpha, PDGFR beta, FGFR1 rearrangements, BCR-ABL, and inv.(16). A definite diagnosis of AML t(8:21) and hypereosinophilia with dysplastic features was then made in combination with cytogenetics.
Discussion: Eosinophilia is a common presentation of underlying disease and often masquerades underlying inflammation, autoimmune disease, malignancy and hematological malignancy. The association of Acute myeloid leukemia with eosinophilia with t(8:21) is rare and hence we reported this case.

A Study on Outcome of Patients With Early T Cell Precursor Acute Lymphoblastic Leukaemia (ETP-ALL) Treated With BFM 2002 Protocol as Frontline Therapy in a Tertiary Care Hospital

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Introduction: Early T-Precursor Acute Lymphoblastic Leukaemia (ETP ALL) is a recently described rare, aggressive and extremely fatal subgroup of T-Acute Lymphoblastic Leukaemia, identified by a distinct gene expression profile and immunophenotype. ETP cells derive from immature hematopoietic progenitor cells with maturation arrest at a very early stage and retain the ability to differentiate into T-cell and myeloid lineage. It's clinical characteristics, treatment, prognosis are significantly heterogenous and the optimal therapeutic approaches are poorly characterized.
Aims & Objectives: To study the outcome among ETP ALL patients treated with BFM 2002 protocol as frontline therapy.
Materials & Methods: A prospective interventional study done in ETP ALL patients aged 5 to 40 years after taking informed consent, diagnosed by bone marrow aspiration and immunophenotyping (CD1a -,CD8-, CD5 dim and positivity for 1 or more stem cell or myeloid antigens) admitted in hematology ward of NRSMCH during the study period september 2021 to august 2022. All received induction therapy as per BFM 2002 ALL protocol.
Result: Seven patients with median age 13 years were included in the study of which four were male and three female. One patient had day 8 good prednisolone response but M3 marrow on day 15, rest six patients had day 8 poor prednisolone response. Three patients each had M3 marrow and M2 marrow on day 15. Three patients achieved complete hematological remission after induction phase A, three patients succumbed due to persisting cytopenias and one patient expired due to febrile neutropenia. Two patients are in consolidation phase as per high risk BFM 2002 protocol. One patient was on maintenance phase but expired after three months due to miscellaneous cause unrelated to disease.
Conclusions: ETP-ALL represents a high-risk disease subtype of ALL. BFM 2002 High risk protocol is inadequate for treatment and there is an urgent need of new tailored therapeutic strategies for the treatment of this disease.

Prognostic Relevance of CD20 Expression Among Pediatric Precursor B-Lineage Acute Lymphoblastic Leukemia Patients

Karthik Bommannan BK, Jhansi Rani A, Venkatraman Radhakrishnan, T.G. Sagar, Shirley Sundersingh

Introduction: CD20 is a surface antigen expressed exclusively among cells of B-lymphoid lineage. CD20 is expressed in nearly 50% of adult precursor B- acute lymphoblastic leukemia patients (B-ALL) and is associated with early relapse and inferior overall survival. Literature on the frequency and prognostic relevance of CD20 expression among pediatric B-ALL patients are both sparse and contradictory.
Methodology: Baseline laboratory characteristics, end of induction measurable residual disease (EOI-MRD) status and survival among pediatric B-ALL patients diagnosed between January 2018 to February 2022 at our institute were retrospectively analysed. Patients with CD20 expression in $> 20\%$ B-lymphoblasts assed by flow cytometric immunophenotyping (FCM) were considered CD20 expressers (CD20 +).
Results: Among 224 treatment naïve B-ALL patients diagnosed during the study time frame, 50% (n = 111) had CD20 expression. Among these CD20 + B-ALL patients, a median (*IQR*) of 67% (*39–91*) blasts had CD20 expression. The clinical and laboratory characteristics among our CD20 + and CD20- B-ALL patients is depicted in Table 1.
Among 211 patients who were treated, 6 patients died during induction. Among the remaining 205 patients, there was no significant difference in the day 8 circulating blast clearance and FCM EOI-MRD status with respect to CD20 expression (refer Table 3). During follow up, there was significant relapse among our CD20 + B-ALL patients as compared to CD20- B-ALL patients (22% vs 9%, p = 0.009). By Kaplan Meier survival analysis, there was significant difference in the 4-year relapse free survival (62% vs 75%, p = 0.011), but not in the overall survival (86% vs 85%, p = 0.887) between our CD20 + and CD20- B-ALL patients. On univariate analysis, expression of CD20 conferred increased risk for poor 4-year relapse free survival (HR: 2.65 with 95% CI of 1.218–5.76, p = 0.014) but did not influence the overall survival (HR: 0.940 with 95% CI of 0.399–2.213, p = 0.887).
Conclusion: CD20 was expressed in 50% of our pediatric B-ALL patients. Baseline clinical and laboratory parameters and EOI MRD were not significantly different with respect to CD20 expression. However, expression of CD20 was associated with significant disease relapse and inferior 4-year relapse free survival.

Moving Towards Chemotherapy Free Management in Acute Promyelocytic Leukemia: Slowly but Surely

Sundar Popat Shewale, Mipsang Lama, Vivek Radhakrishnan, Anjali Deshmukh, Vasundhara Raina, Arijit Nag, Rizwan Javed, Saurabh Bhave, Jeevan Kumar, Mayur Parihar, Sushant Vinarkar, Deepak Mishra, Mammen Chandy, Reena Nair

Introduction: Acute Promyelocytic Leukemia (APL), is a paradigm of successful targeted therapies. Clinical observations, followed by understanding the disease biology laid the foundation for therapeutic improvement over the last three decades.
Aims & Objectives: Audit of APL patients treated during the last decade as treatment changed from chemotherapy-based regimens to targeted treatments.
Materials & Methods: A retrospective analysis of 82 APL patients treated between August 2011 to May 2019. Patients were risk stratified according to Sanz score. Patients were categorised in three groups depending on the therapeutic regimen used.

Group 1: Single agent ATO (Arsenic trioxide) ± chemotherapy.
Group 2: ATO + ATRA (All trans retinoic acid) ± chemotherapy.
Group 3: Single agent ATRA + chemotherapy (Lo- Coco protocol).

In Group 1 and 2 chemotherapy was given only to treat hyperleukocytosis. Patients in complete remission (CR), received consolidation with ATO and ATRA for 28 days followed by maintenance ATO/ATRA 10 days a month for 6 months.
Result: The median age of the patients in this cohort was 37 years (range 18–67), with predominance of females (54%). The distribution of patient into high, intermediate and low risk as per the Sanz score was observed as 46%, 45% and 9%, respectively. Majority of patients received ATO + ATRA ± chemo (60%) as induction regimen, followed by ATO ± chemo (35%) and ATRA ± chemo (5%).
After a median follow up of 36 months, 14 events (17%) were recorded. 10 were early deaths within a month of diagnosis. 7 patients died with disease secondary to sepsis and haemorrhage, 2 of sepsis and 1 cardiac cause. 4 patients relapsed of which 2 were salvaged with second line therapy.

Survival analysis demonstrated 100% probability for OS and EFS for low-risk patients, and the survival probability for high (OS = 75%, EFS = 75%) and intermediate (OS = 85%, EFS = 80%) risk was lower.

Table 1 Clinical and laboratory profile of pediatric B-ALL patients with and without CD20 expression

Parameters	All patients (n = 224)	CD20 expression		P value
		CD20- (n = 113)	CD20 + (n = 111)	CD20- vs CD20 +
Median (range) age in years	5 (1–18)	5 (1–18)	5 (1–18)	0.972
Sex (Male: Female)	1.5:1	1.35:1	1.7:1	0.397
Median (range) Hb in g/L	70 (22–149)	66.5 (22–122)	71 (25–149)	0.161
Median(range) WBC count $X10^9$/L	9.6 (0.3- 587)	10.7 (0.7 to 587)	8.2 (0.3 to 216)	0.203
Median (range) Platelet $X10^9$/L	45 (4–563)	50 (4–563)	39 (5–369)	**0.023**
Median (range) PB blasts %	54 (0–98)	60 (0–98)	45 (0–96)	**0.037**
Median (range) BM blasts %	90 (25–99)	90 (28–99)	90 (25–99)	0.685
Splenomegaly (%)	51%	49	53	0.514
Hepatomegaly (%)	43%	38	49	0.111
Lymphadenopathy (%)	49%	46	53	0.321
CNS involvement at diagnosis (%)	2%	1	3	0.308
Ploidy (%)				
Diploid	71% (150 of 212)	70 (76 of 109)	72 (74 of 103)	0.638
High Hyperdiploid	21% (44 of 212)	24 (26 of 109)	18 (18 of 103)	
Low Hyperdiploid	5% (11 of 212)	5 (5 of 109)	6 (6 of 103)	
High Hypodiploid	1% (1 of 212)	0 (0 of 109)	1 (1 of 103)	
Near Triploid	1% (2 of 212)	1 (1 of 109)	1 (1 of 103)	
Near Tetraploid	2% (4 of 212)	1 (1 of 109)	3 (3 of 103)	
NCI High risk (%)	42% (93 of 224)	35% (40 of 113)	48% (58 of 111)	0.061
BCR-ABL1 fusion positive (%)	5% (11 of 212)	4% (4 of109)	7% (7 of 103)	0.305
ETV6-RUNX1 fusion positive (%)	10% (22 of 212)	13% (14 of 109)	8% (8 of 103)	0.226
KMT2A rearranged (%)	2% (5 of 212)	4% (4 of 109)	1% (1 of 103)	0.196
TCF3-PBX1 fusion positive (%)	7% (14 of 212)	7% (8 of 109)	6% (6 of 103)	0.657
High Risk cytogenetics (%)	10% (22 of 212)	9% (10 of 109)	12% (12 of 103)	
Induction death (%)	3% (6 of 211)	2% (2 of 106)	4% (4 of 105)	0.401
D8BNC (%)	10% (20 of 211)	13% (14 of 106)	6% (6 of 105)	0.063
End induction MRD positive (%)	38% (77 of 203)	36% (37 of 104)	40% (40 of 99)	0.479
All Relapse (%)	15% (31 of 205)	9% (9 of 104)	22% (22 of 101)	**0.009**
4-year				
Overall Survival	85.3%	85%	85.6%	0.887
Relapse Free Survival	68.4%	75.4%	62%	**0.011**

B-ALL: B lineage acute lymphoblastic leukemia; BM: Bone marrow; CNS: central nervous system; D8BNC: Day 8 blast not cleared; IRQ: Inter quartile range; MRD: minimal residual disease; NA: not applicable; NCI: National cancer institute; PB: Peripheral blood; WBC: white blood cells; High Risk cytogenetics:

ATO + ATRA ± chemo group showed good survival probability with an OS and EFS of > 90% compared to the ATO ± chemo (OS = 73% p = 0.012, EFS = 65% p = 0.008).

Cox multivariate model predicted ATO ± chemo, ICU stay as associated with inferior prognosis.

Conclusions: Treatment of APL patients confirms high survival rate with targeted ATO + ATRA ± chemotherapy. Treatment with single agent ATO ± chemo and ICU stay are independent prognostic markers and associated with inferior prognosis.

Adolescent and Young Adult Acute Lymphoblastic Leukemia: Real-World Data from a Tertiary Care Center in North India

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Introduction: Adolescents and Young Adults with acute lymphoblastic leukemia (AYA-ALL) have improved outcomes by pediatric or pediatric-inspired protocols. The results published from

trials are different from population-based studies, that too in the real-world settings with resource constraints.
Aims & Objectives: We aimed to study the characteristics and outcomes of AYA-ALL in real-world settings.
Materials & Methods: It is a retrospective observational study involving all patients of AYA-ALL (aged 15-39y) managed at a tertiary care center in North India over 07 years. Survival outcomes of AYA-ALL are based-on age, gender, risk stratification, relapse status, CNS involvement, type of protocol, and phenotypic classification. Data were analyzed using JMP and R software.
Result: AYA-ALL constituted 18.98% (116) of ALL patients managed in the study period. The mean age of the study group was 26.65 ± 6.61 years (range 16–39) with 32.7% (n-78) being males. On risk stratification, 26 (22.4%) and 23 (19.8%) patients were classified as intermediate and high risk respectively. The cumulative overall survival at 1y, 3y, and 5y were 86.98%, 67.9%, and 55.6% respectively. Patients managed with adult GMALL protocol had better survival whereas those on Hyper-CVAD therapy had the least survival (p-0.0011). The survival was also significantly different between different risk groups, relapsed disease, and poor cytogenetics. The survival was not statistically different stratified by Ph-positivity, gender, and B/T immunophenotype.
Conclusions: Adult GMALL protocol had better survival vis-à-vis pediatric BFM protocol in our study cohort owing to less toxicity. Intensive pediatric/pediatric-inspired protocols in resource constraint settings can lead to poorer outcomes due to suboptimal care for higher toxicity.

Sequential Bilateral Bell's Palsy in a Child with Acute Lymphoblastic Leukaemia: Is It Smouldering Fire??

Naga Geetha Rani Mangam, Julius Xavier Scott, Dhaarani Jayaraman, Padmasani Venkataraman, Deepthi

Introduction: Peripheral type facial nerve paralysis (Bell's palsy) is mainly seen clinically as the idiopathic type. Bell's palsy in a child with leukaemia often is attributed to infections or a relapse. We present a girl with sequential bilateral Bell's palsy without an obvious infection/relapse.
Aims & Objectives: Case details:- Miss. A, 9 years girl was diagnosed with B-Acute lymphoblastic leukaemia without any high-risk cytogenetics and no CNS disease at diagnosis. She had poor prednisolone response and MRD was negative < 0.01% at the end of induction.
Materials & Methods: Post induction chemotherapy, a week later, she was brought with acute onset of deviation of angle of mouth to left and inability to close right eye. MRI Brain, MRV and MRA were normal. CSF showed no cells/albuminocytological dissociation or malignant cells. Viral PCR studies in CSF including EBV, herpes and CMV PCR were negative. She was diagnosed with Right Bell's Palsy and was started on steroids and Acyclovir. Physiotherapy was also suggested appropriately. She was continued on chemotherapy after recovery and 4 weeks later, she developed Left Bell's palsy after week 9 of consolidation therapy. Repeat CSF analysis was negative for viral PCR and malignant cytology.
Result: She was managed conservatively and vincristine doses were titrated and resumed after clinical recovery. Currently, she has mild residual palsy on left side and normal function on the right side at the end of intensive phase chemotherapy.
Conclusions: Cranial nerve palsies including Bell's palsy have always been described in association with relapse in patients with ALL. Idiopathic sequential bilateral facial palsy in the absence of relapse or infection has not been reported in literature. Possibility of drug toxicity remains a clinical concern.

Peg-Asparginase Induced Hypoglycaemia: A Case Series

Juhi Mehrotra, Santanu Sen

Introduction: PEG-asparaginase is a modified version of L-asparaginase derived from E.coli, which is an integral component of Paediatric ALL treatment. Hypersensitivity, coagulopathy, pancreatitis and hyperglycemia have been noted as common side effects.
Aims & Objectives: Herein, we report a case series of hypoglycemia associated with Peg-asparaginase.
Materials & Methods: Three cases on UKALL induction chemotherapy with Dexamethasone at 10 mg/m2/day (D 1 to D 14), Vincristine and Daunorubicin on D 1, 8, 15, 22 and 29 and Peg asparaginase on D 4 and 18, maintained normal blood sugars during the first 2 weeks and had asymptomatic hypoglycaemia ranging from 54 to 60 mg/dl, 3–5 days post second dose of peg asparaginase on day 18. Critical sampling was done and pancreatitis with other metabolic causes of hypoglycaemia were ruled out. They were maintained on GIR of 12–15 for the next week and started on oral complex starch and monitored closely.
Result: No hypoglycemia was noted post first dose of peg asparaginase, while the patients were on Dexamethasone. We observed, the first hypoglycemia on day 20–23 after the second dose of Peg asparaginase on day 18, while dexamethasone was stopped. Given the similar temporal association between the peg asparaginase and hypoglycemia observed in all three children, hypoglycemia can be associated with peg asparaginase.
Conclusions: Surprisingly normal circulating insulin levels, free fatty acids, and no ketone bodies were noted during hypoglycemia, confirming hyperinsulinism as a possible cause. Concomitant use of glucocorticoids during induction therapy for childhood ALL may mask the hypoglycemic effect of L-Asp due to steroid induced hyperglycemia. This may be the reason that hypoglycemia has not been reported. We wish to highlight the importance of carefully monitoring these group of patients for hypoglycemia in addition to its better known adverse effects.

Frequency of Genetic Abnormalities by Conventional Karyotyping & Multiplex Pcr in Pediatric & Adolescent Acute Lymphoblastic Leukemia: A Single-Centre Experience

Jasmine Porwal, Anamika Bakliwal, Karthik Kumar, Sashi Kant Singh, Jhasaketan Nayak, Vinod Kumar, Gaurav Dhingra, Uttam Kumar Nath

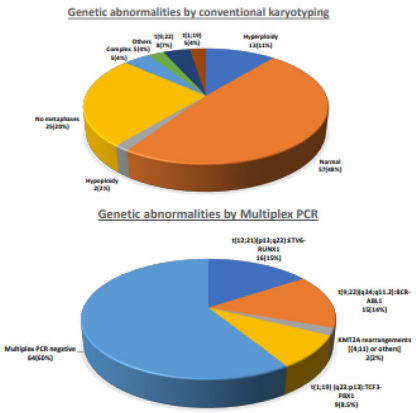
Introduction: Acute lymphoblastic leukemia (ALL) is the commonest childhood cancer. Risk stratification at diagnosis is based on clinical parameters and detection of genetic abnormalities by different techniques with varying diagnostic yields. Availability of molecular techniques like multiplex PCR & next-generation sequencing (NGS) have provided major insights into disease biology and risk stratification of pediatric& adolescent ALL.
Aims & Objectives: The objective of this study is to describe the frequency of genetic abnormalities detected by conventional karyotyping & multiplex PCR techniques in pediatric& adolescent ALL patients treated at AIIMS Rishikesh.
Materials & Methods: We analysed data of 118 newly diagnosed ALL patients in the age group of 1–24 years treated between February 2018 & August 2022. After confirmation of ALL diagnosis by immunophenotyping, bone marrow cytogenetics (by G-banded karyotyping) & peripheral blood multiplex PCR panel for recurrent genetic abnormalities were sent for baseline risk stratification. All the patients were treated with ALL IC-BFM 2009 protocol. Other genetic tests like NGS and/or FISH for Ph-like ALL panel (ABL1, ABL1, CRLF2, JAK2, EPOR1, PDGFRA/B, & CSF1R rearrangements) could be done only in a few patients.

Result: Data of 118 newly diagnosed pediatric& adolescent ALL patients [B-ALL = 90 (76%), T-ALL = 28 (24%)] were analysed. Median age was 8.5 years (range 1–24 years). The commonest abnormality detected by karyotyping was hyperdiploidy (11%). The commonest genetic abnormality detected by multiplex PCR was t(12;21)(p13;q22):ETV6-RUNX1 (15%), followed by t(9;22)(q34;q11.2):BCR-ABL1 (14%) and t(1;19)(q23;p13.3):TCF3-PBX1 (8.5%). Other genetic abnormalities included t(4;11)/KMT2Ar variants, t(2;11), t(2;16), del(5)(q31q35) and trisomy 8. Conventional karyotyping & multiplex PCR failed to detect any genetic abnormality in almost 50% patients in our study. Abnormalities like KRAS, SETD2, ETV6 & TP53 mutations were detected by NGS.
Conclusions: Our experience of genetic risk stratification in pediatric& adolescent ALL patients by using conventional karyotyping & multiplex PCR panel for recurrent genetic abnormalities suggests that although these techniques are adequate for identification of commonly occurring genetic abnormalities, incorporation of other techniques like NGS and FISH for Ph-like ALL at baseline are expected to identify additional genetic alterations and further improve the risk stratification for refinement of prognostication & tailoring treatment strategy.

Table 1: Genetic abnormalities in pediatric & adolescent ALL patients in our study:

No. of patients	Age (years)	Gender	Conventional Karyotyping [n = 118]	Multiplex PCR panel [n = 106]
118	Median age: 8.5 (IQR: 4-16) years	Male: 70 (59%) Female: 48 (41%)	Normal karyotype = 57 (48%) Hyperdiploidy = 13 (11%) t(9;22) = 8 (7%) t(1;19) = 5 (4%) t(4;11)/variant = 2 (2%) Complex karyotype = 5 (4%) Hypoploidy = 2 (2%) Other abnormalities = 5 (4%) No metaphases = 25 (20%)	t(12;21):ETV6-RUNX1 = 16 (15%) t(9;22):BCR-ABL1 = 15 (14%) t(1;19):TCF3-PBX1 = 9 (8.5%) t(4;11)/KMT2Ar variants = 2 (2%) Multiplex PCR negative = 64 (60%)

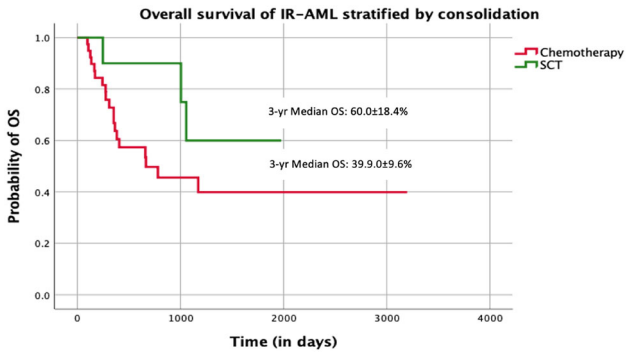
Figure 1: Frequency of genetic abnormalities by Conventional Karyotyping & Multiplex PCR



Real World Outcomes in Intermediate Risk AML: Data from a Tertiary Care Center in Eastern India

Arjin Philips Jacoby, Rajat Pincha, Arijit Nag, Vivek Radhakrishnan, Saurav Bhave, Jeevan Kumar, Deepak Mishra, Mayur Parihar, Reena Nair, Mammen Chandy

Introduction: Acute myeloid leukemia (AML) is a life-threatening hematological malignancy with poor long term outcomes. There is paucity of real-world survival data from the Indian sub-continent.
Aims & Objectives: To analyse overall survival (OS) in intermediate risk AML (IR-AML).
To describe demographic and molecular profile in these patients.
Materials & Methods: This was a retrospective descriptive study done at Tata Medical Center, Kolkata. A total of 85 patients with intermediate risk AML (ELN 2017), treated at our center were included in the study from January 2011 to December 2020. Demographic details, clinical presentation, molecular/cytogenetic profile, treatment and survival outcomes were analyzed. Descriptive statistics and survival analysis were done using Microsoft Excel and SPSS-version 26 respectively.
Result: Intermediate risk AML was more common in males (55.3%), between age 35–59 years (61.2%) and presented with ECOG 1 (73%) [Table 1]. Of the 85 patients, 80% (n = 68) received 3 + 7 Induction chemotherapy while 20% (n = 17) were given hypomethylating agents. Response assessments were available for 67 patients of which 67.2% had remission following 1st Induction. 11 patients underwent 2nd induction of whom 54.2% (n = 6) went into remission. Consolidation was only chemotherapy in 79.6% (n = 39) patients whilst allogenic stem cell transplantation (SCT) was done in 20.4% (n = 10). Amongst 42 patients receiving consolidation chemotherapy, high dose [HIDAC] (3 g/m2) vs Intermediate dose [IDAC] ($\leq$ 1.5gm/m2) cytarabine were given in 19% (n = 8) and 81% (n = 34) respectively with no significant survival difference in 3-year median OS (IDAC: 42.65% Vs HiDAC: 43.75%). 76.2% (n = 32) cases received all 3 cycles of chemo-consolidation. 30.6% patients relapsed with a median duration of 229 days, of which 73.1% (n = 19) relapsed post consolidation. Median overall survival (OS) of the entire cohort was 382 days (95% CI: 269–781). The median OS at 3 years was 45.6% (95% CI: 27.5–62%) vs 60.0% (95% CI: 19.0–85.5%) in chemotherapy vs SCT groups, respectively (p = 0.116).
Conclusions: Survival outcomes were similar for IDAC vs HIDAC chemotherapy. Survival outcomes were significantly better post SCT in comparison to chemotherapy alone, and should possibly be the consolidation of choice in IR-AML.



Venetoclax and Azacitidine as a First Line Therapy in AML

Harshwardhan Bahirat, Priyanka Samal, Anindita Paul

Introduction: The Complete remission rate achieved by conventional chemotherapy (7 + 3) in patients > 40 yrs is only 31%. Even Less intense approaches are associated with poor response rates of < 30%. Venetoclax in combination with Azacitidine for transplant ineligible patients was approved by the US FDA in 2018 as a new line of therapy. Here we report our single-center experience with Venetoclax and Azacitidine in 19 AML patients who were not eligible for standard induction therapy.

Aims & Objectives: The primary objective of the study was to assess efficacy (CR + Cri + PR) of the regimen. Major adverse effects and duration of response achieved were also studied.
Materials & Methods: Azacitidine at a dose of 75 mg/m2 per dose was administered for 7 consecutive days on days 1–7, every 28 days/cycle till disease progression. Venetoclax 100 mg daily was started on along with D1 Azacitidine and was continued for 28 days. Posaconazole prophylaxis 300 mg daily was added. Response assessment following 2 cycles, and then every 3–4 cycles or as indicated clinically with CBC with differential, bone marrow aspirate, and biopsy was performed.
Result: The median age of the patients was 58 years (47–80 years) with M:F ratio of 10:9. The median follow-up was 6.5 months (0.5–24 months). The median no of cycles received was 4 (1–21). Therapeutic response assessment could be done in 16 (84%) patients after 2 cycles of therapy. Response (CR + Cri + PR) was achieved in 13 (68.4%) patients. Dose reduction was required in 7/9 patients who received ≥ 4 cycles (36%). 3 (15.7%) patients relapsed after a median of 9 months. The most common adverse effect documented was grade II cytopenia observed in 9 (47.3%). 6 (31%) patients expired during the course of disease with 5 patients dying due to sepsis and 1 patient due to chronic SDH (in view of platelet refractoriness). 1 patient was lost to follow-up.
Conclusions: Our data confirms that Venetoclax and Azacitidine based regimen is a viable and safe option for AML patients unfit for intensive chemotherapy.

Core Binding Factor AML: Is It Good Risk!!!—Real World Data on Survival Outcomes from a Tertiary Care Center in Eastern India

Debranjani Chattopadhyay, Sutapa Chatterjee, Arijit Nag, Saurabh Bhave, Jeevan Kumar, Mayur Parihar, Deepak Kumar Mishra, Reena Nair, Mammen Chandy

Introduction: The Core Binding factor Acute Myeloid Leukaemia (CBF-AML) is a favorable risk AML as per the ELN 2017 classification and includes either t(8;21) (q22;q22) or inv(16) (p13q22)/t(16;16). There is an acute paucity of published data on the survival outcomes of CBF-AML in India.
Aims & Objectives: Our study aims to describe the clinico-demographical characteristics of patients from the Indian sub-continent with CBF-AML and to evaluate the outcomes in terms of morphological complete remission rates (CR), overall survival (OS), and disease-free survival (DFS) following treatment.
Materials & Methods: Ours is a retrospective descriptive study done at Tata Medical Centre, Kolkata, between January 2011 and December 2021. We enrolled 51 patients with CBF-AML (ELN 2017) treated at our center, of which 45 patients were studied for their demographic and clinical profile, Laboratory profiles, treatment, and survival outcomes following chemotherapy. Descriptive statistics and survival analysis were done using Microsoft Excel and SPSS-version23 respectively.
Result: The baseline characteristics and treatment details in our study cohort are outlined in Table 1 and Table 2 respectively. Five patients (11%) received HMA (Hypomethylating agents) before induction owing to sepsis at presentation. 41 patients (91%) received 3 + 7 chemotherapy, and 4 received HMA-based induction. 30 patients (66%) received the full dose of 3 + 7 chemotherapy, dose was reduced in the rest owing to complications. 31 patients were in CR (69%) at the end of induction. Consolidation chemotherapy was given to 31 patients. Following intensive chemotherapy (induction + consolidation) 13 patients (28%) expired, 2 relapsed, 1 was lost to follow up and 29 (64%) were in CR. The median follow-up duration was 326 days. 13 patients (28%) relapsed at 2 years, and the median time to relapse was 385 days. At 2 years, the median overall survival (OS) was 53.7% (95% CI: 44.5 to 62.9%) and the disease-free survival (DFS) was 43.6% (95% CI: 34.2 to 53%).
Conclusions: Our study is a retrospective study with its inherent shortcomings. Although CBF AML is considered a good-risk AML subgroup, we have an OS of 53% and DFS of 43.6% at the end of 2 years follow-up. Our survival rates are still low probably due to the combined effect of higher rates of sepsis-related mortality, treatment abandonment & relapse.

Table 1: Baseline characteristics

Variable	Frequency (%)
Demographic Profile	n=45
Age Group	
18-34	19 (42.2)
35-59	23 (51.1)
> 60	3 (0.06)
Gender	
(Male)	24 (53.3)
(Female)	21 (46.7)
ECOG	
1	27 (60)
2	11 (24.4)
3	7 (15.6)
Co-morbidities	
DM	6 (13.3)
HTN	2 (4.4)
Hypothyroidism	2 (4.4)
Others	3 (6.6)
No comorbidities	31(70.5)
Clinical Presentation	
Fever	28 (62.2)
Bleeding	17 (37.8)
Spleen	3 (6.7)
Liver	3 (6.7)
LNE	3 (6.7)
Pain	8 (17.8)
Weight loss	5 (11.1)
Weakness	31 (68.9)
Dyspnoea	12 (26.7)
Laboratory Profile	
Immunophenotype	
Aberrant CD19+/CD56+	16 (28.5)
Karyotype	
t(8;21) (q22;q22)	31(68.8)
inv(16) (p13q22)/t(16;16)	14 (31)
Asso. Karyotype anomaly	
sex chromosome loss	19(42.2)
trisomies	14(31.1)
monosomies	1 (0.02)
complex karyotype	1 (0.02)
Molecular Profile	
FISH cKit	9(37.5)
PCR/ Sanger	
NPM1	0
FLT3 ITD	1
FLT3 TKD	0
NRAS	1

Table 2: Treatment Profile

Variable	Frequency (%)
Induction Chemotherapy (n=45)	
Pre-induction HMA	5 (11.1)
3+7	41 (91.1)
Full dose delivered	30 (66.7)
HMA (Decitabine/Azacytidine)	4 (8.8)
Post Induction Response (n=45)	
Complete remission	31 (67.2)
Not in remission	2 (4.4)
Death	10 (22.2)
LTFU	2 (4.4)
Consolidation Therapy (n=31)	
HIDAC	10 (32.3)
IDAC	19 (61.3)
IDAC + GO	1 (3.2)
HMA	1 (3.2)
Relapse	13 (28)
Median duration of relapse	385 days
Time point of relapse	
Post Induction	0
During Consolidation	2 (4.4)
Post-treatment	
<6 months	1 (2.2)
>6 months but < 1 year	6 (13.3)
>1 year	4 (8.8)
Post relapse Treatment	13(28)
Palliation	11(84.6)
Induction 2 f/b HSCT	4 (30.7)
Type of Transplant	
Matched Sibling donor	1
Matched Related donor	1
Haploidentical donor	1
Mismatched unrelated donor	1
Post-transplant outcome	
Remission	1
Death	3
Status at last follow up	
Alive in CR	16 (35.5)
Dead	17 (37.7)
Alive with Disease	10 (22.2)
LTF	2 (4.4)

Predictive Factors for High Dose Methotrexate Induced Chemotoxicties in Newly Diagnosed Acute Lymphoblastic Leukemia: A Tertiary Center Experience from Lower-Middle-Income Country

Gopinathan Mathiyazhagan, Anshul Gupta, Sanjeev, Soniya Nityanand, Rajesh Kashyap, Ragavendra L

Introduction: High dose methotrexate (HD-MTX) chemotherapy infusion given as extracompartmental therapy to newly diagnosed Acute Lymphoblastic Leukemia (ALL) patients aids in eradication of leukemic deposits at sanctuary sites. The challenge of delivering HD-MTX in LMIC like India lies in addressing decreased patient to bed ratio, waiting time for admission, access to TDM, and providing supportive care for chemotoxicities with limited financial support.
Aims & Objectives: To analyse the various patient and disease related predictive factors to the occurrence of HD-MTX induced chemotoxicity.
Materials & Methods: The study was a prospective observational study carried out in the Department of Hematology at a North Indian tertiary care centre from January 2021 to February 2022. Chemotoxicities such as mucositis, hematological toxicities, AKI, transaminitis, hyperbilirubinemia, febrile neutropenia noted after HD-MTX infusion were recorded and graded as per the National Cancer

Institute Common Toxicity Criteria (CTCAE version 4.0) during the inpatient stay and every 3rd day after discharge during the routine outpatient visit. They were correlated with various patient factors like age, gender, risk category of ALL, presence of malnutrition, prior induction chemotoxicity, and serum albumin levels before initiation of each HD-MTX cycle by univariate binary logistic regression analysis. Multivariate analysis was applied to those parameters which were significant in univariate analysis.

Result: A total of 69 patients with a median age of 12 (2–35) years who received 267 HD-MTX cycles were analyzed. Hematological toxicities were most common followed by mucositis with grade III-IV neutropenia, anemia, thrombocytopenia, mucositis noted in 50.5%, 18.2%, 17.6%, 13.1% of them. On logistic regression analysis higher 36 h S.MTX levels (> 5 mM/L), first 2 cycles of HD-MTX, presence of hypoalbuminemia (< 3 g >

Conclusions: HDMTX (5gm/m2) can be safely administered with minimal therapeutic drug monitoring in resource constraint settings. In addition to serum MTX levels, timely identification of various patient and disease related risk factors is of vital importance in order to circumvent MTX toxicity and treatment cost.

To Study the Cost Effectiveness Of RDP (Any Group with Any Rh Type) in Maintaining Hemostasis in AML (Other Than APML) Patients Receiving Induction 3 + 7 Chemotherapy (CT)

Anwesha Mohanty, R.K.Jena

Introduction: Patients with AML develop bleeding manifestations due to CT induced thrombocytopenia and underlying disease and complications. This complication puts a lot of challenge in the management of these patients and can lead to significant morbidity and mortality which can be managed either with Single donor platelet (SDP) or random donor platelet (RDP). The former is expensive and challenging to the blood bank inventory system while the latter is cheap, easily available and users friendly albeit less effectiveness due to alloantibody production (multiple donors, ABO/Rh incompatible). The following study evaluates the safety and effectiveness of RDP In AML receiving induction CT.

Aims & Objectives: To study the cost effectiveness of RDP (any group with any Rh type) in maintaining hemostasis in AML (other than APML) patients receiving induction 3 + 7 chemotherapy (CT).

Materials & Methods: Prospective study including 105 confirmed cases of AML being treated in clinical hematology department of SCB MCH, Odisha.

INCLUSION CRITERIA-

- AML patients diagnosed by flow cytometry and other parameters.
- Age ≥ 14 years ≤ 60 years.

EXCLUSION CRITERIA-

- APML patients.
- AML patients with significant co-morbidity like diabetes, IHD, renal impairment (Creatinine ≥ 2 mg/dl), liver dysfunction (bilirubin ≥ 2).

INVESTIGATIONS-

- CBC
- Kidney function test.
- Liver function test.
- PT, Aptt, INR.
- Fibrinogen.
- D-Dimer.

TREATMENT:

- Induction CT as 3 + 7; Daunorubicin @ 60 mg/m^2/day for D1-D3 and ARA-C 100 mg/m^2/day from D1-D7.
- Other supportive therapy as per SOPs.
- RDP 4–6 units (irrespective of ABO group and Rh status) were administered daily from D1 and was titrated depending on target TPC (> 10,000/mm^3) and/or bleeding manifestations till bone marrow recovery.

The 105 patients included under this study undergo the above investigations at their baseline on the day of starting induction CT and thereafter monitored with CBC every day, coagulation profile every 3rd day and also looking for any bleeding manifestations and any adverse reactions to RDP received for 2 weeks.

Result: TPC on D1(median)—10,000(3000–26,000).

TPC on D15(median)—30,000(20,000–42,000).

Average no of RDP—66.

Adverse reactions-

- None- 24.7%
- Minor- 68.4%
- Major- 6%

Bleeding manifestations-

- None- 14.2%
- Minor-72.3%
- Hemorrhage requiring RBC support- 9%
- Life threatening- 3%

Conclusions: With 66 units of RDP on average, satisfactory hemostasis in 97% of patients.

- Adverse reactions were detected in 74% percent cases, fever, chills and rigor are common and could be managed with supportive therapy in all cases.
- The procurement of RDP was easy (as it was any group with any Rh status) and the administration was users friendly in all cases.
- RDP is safe and effective with advantages of cost and easy availability which is beneficial in resource limited health institutions as well as for the people belonging to low socio-economic status.

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	Total cost of RDP	Estimated cost of SDP
TPC range	30000 - 260000	35000 - 330000	60000-300000	84000-260000	40000-270000	80000-300000	95000-350000	120000-330000	100000-320000	120000-280000	150000-320000	160000-350000	135000-330000	150000-400000	200000-420000		
TPC Median ~	10000	10000	13000	17000	10000	12000	17000	25000	20000	17000	22000	20000	22000	25000	30000		
PT- INR avg	1.45			1.68			1.54			1.74			1.45				
Fibrinogen	540			572			420			324			250				
D-Dimer	870			653.5			492			375			244				
No. of RDP /day	4	4	4	4	6	6	6	6	5	5	4	4	4	2	2	Rs 9240	Rs 1,32,000

Incidence & Clinical Spectrum of Bacterial Infections in Patients of Adult AML (15-60yrs) (Other than AML 3) Receiving Induction Ct (3 + 7) in General Ward

Biswajeet Bahal, J K Panda, S SETHY

Introduction: Acute myeloid leukemia (AML) needs 3 + 7 induction CT. Major complication is myelosuppression, severe bacterial infections increasing both mortality & morbidity. Ideally these

patients should be treated in isolated wards with barrier nursing & all other facilities which are not available in general ward of resource constrained institutions.

Aims & Objectives: To estimate the incidence & clinical spectrum of bacterial infection during induction CT.

Its impact on marrow recovery, CHR & mortality.

Materials & Methods: Its a prospective study involving 72 patients treated in CHW from January 2021- July 2022.

INCLUSION CRITERIA-

- Adult (15-60yrs)
- Confirmed cases of AML (other than AML3)
- Willing to receive 3 + 7 CT

DOCUMENTATION OF BACTERIAL INFECTION-

- Patients were suspected & subjected to further investigations if
- Fever (one temperature > 38.4 oC or 3 readings > 37.9 oC but < 38 >

Symptoms/signs related to specific organ system involvement.

Following investigations were done to confirm diagnosis– CBC, Blood C/S, urine R/M & C/S, sputum C/S, Xray chest & HRCT Thorax, viral & fungal markers (to exclude).

TREATMENT –

Patients with evidence of bacterial infection will receive antibiotics as per institutional SOPs; Cefoperazone + sulbactam with Amikacin or teicoplanin with aztreonam as per the situation. CHR & Mortality were noted in all cases.

EVALUATION:

All the patients were evaluated for Bone marrow recovery, CHR as per standard criteria & were compared between patients with & without bacterial infection. CHR is defined as per standard criteria.

Result: Forty one patients (56.9%) were detected to have bacterial infection out of which 8 patients had mixed infections. The respiratory infection was most common (43%). The bone marrow recovery was delayed (median D29) with high mortality rate (15.2%) in comparison to patients without infection.

Conclusions: Bacterial infections are seen in 56.9

ALL a Great Mimicker!! A Rare Case of ALL Disguised as Neurofibromatosis

Dyvik S, S Vaishnavi D Siyaram S Lokesh T Sarbesh K Daisy, P Abhishek, S Kuldeep

Introduction: Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, characterized by the rapid proliferation of poorly lymphoid progenitor cells in the bone marrow. Being the great mimick it is, ALL has quite a varied presentation at onset and course ahead. We hereby present a case of ALL incidentally diagnosed while working up for neurofibromatosis. Further ahead into the treatment, this case also showed features of intrathecal methotrexate-induced leukoencephalopathy, which is quite a well-known complication in such genetic cases except for the fact that these features developed in our child after just the first dose of intrathecal methotrexate.

Aims & Objectives: The aim of presenting this case report is to highlight keeping a high index of suspicion for treatable acquired causes like acute leukemia, which can have varied presentations mimicking any genetic or acquired chronic or acute condition. We must do appropriate and detail clinical and laboratory evaluation if the case has any atypical clinical features even with associated genetic conditions hence timely diagnosis and treatment can be possible to confirm the diagnosis and avoid unnecessary morbidity and mortality along with wastage of resources.

Materials & Methods: The Index Case was evaluated and managed by an integrated hematology team of pediatrichematology, pediatric neurology, intervention, and diagnostic radiology and the Department of Pathology & Lab Medicine at All India Institute of Medical Sciences, Jodhpur. The Boy was admitted given the child presented to our emergency department with complaints of chronic fever and generalized swelling with facial puffiness. On evaluation, the child was to be having a progressive hearing loss for the last 3 months and evaluated with complete history, sequential haemogram findings, peripheral smear examination, morphology, detailed coagulation study, and bone marrow examination along with molecular testing to confirm the diagnosis.

Result: A 9-year boy presented to us with complaints of chronic fever and generalized swelling with facial puffiness. On evaluation, the child had a progressive hearing loss for the last 3 months. Examination revealed pallor, generalized edema, periorbital puffiness, multiple cafe- au-lait spots all over the body, prominent right costochondral junction, and B/L ballotable mass palpable in the abdomen. Initially, the child was then worked up about the possibility of neurofibromatosis. Ultrasound abdomen showed B/L renomegaly with mild hydronephrosis. MRI brain and spine showed changes suggestive of leukemic encephalopathy with vertebral enhancing lesions. Clinico Radiological diagnosis of neurofibromatosis 1 was made, and the child was worked up further in the direction of MRI leukemic findings. Blood film morphology showed microcytic hypochromic anemia with a few target cells and pencil cells. Bone marrow biopsy revealed diffuse marrow infiltration by blasts suggestive of acute leukemia. Further cytogenetics and flow cytometry was suggestive of B cell acute lymphoblastic leukemia.

Conclusions: Child The index highlighted again that hematologic malignancy could present in any form with a very atypical presentation. Hence detailed systemic clinical and diagnostic evaluation can save time and resources and help in early diagnosis for optimal management. Hence chronic case with atypical features needs to be evaluated in a multidisciplinary unit, and timely referral to such a unit with good integration between teams is very important for optimal outcome.

Philadelphia-Positive Acute Lymphoblastic Leukaemia: Demographic And Presentation

Sudip Roy, MaitreyeeBhattachary

Introduction: The Philadelphia (Ph) chromosome is one of the common cytogenetic abnormalities in adult Acute Lymphoblastic Leukaemia (ALL). It is seen in 20–30% of adult cases and 3–5% of paediatric cases. Ph + ALL is known for worse outcome before the use of tyrosine kinase inhibitors.

Aims & Objectives: Demographics and clinical profile of the Ph + ALL patients treated at IHTM in last three years according to modified E- Wall protocol without bone marrow transplantation, compared with available national data.

Materials & Methods: Total 302 ALL patients, age range from 8 months to 56 years, were included in this study who were treated at IHTM from September, 2019 to August, 2022. These patients were categorised as Ph + ve or Ph -ve according to bone marrow aspirate cytogenetics findings and treated according to institutional protocols.

Result: 126 adult and 176 paediatric patients were included in this study and 13 adult (10.31%) and 3 paediatric (1.70%) were Ph + ve

with male (68.73%) predominance. Most of the patients (87.5%) presented with hyperleukocytosis, aberrant expression of CD13 and / or CD33 (56.25%). During induction therapy 12 Ph + ve (75%) and 138 Ph-ve (48%) patients developed febrile neutropenia. 9 of the 13 Ph + ve (69.23%) and 166 of the 176 Ph- ve patients (94.31%) achieved remission after 1st induction therapy.

Conclusions: The overall incidence of Ph + ve ALL in this study population is comparable with published Indian data. But in paediatric age group the incidence is slightly higher. Male sex, hyperleukocytosis and aberrant myeloid markers are the strongest associated factors at presentation.

A Rollercoaster of Challenges: From Diagnosis to Treatment in a Unique Case of CML in Blast Crisis

Aishwarya Keshan, Suchita Shinde, Arjin Philips Jacoby, Vinay Anand, Debranjani Chattopadhyay, Arijit Nag, Sushant Vinarkar, Mayur Parihar, Jeevan K Garg, Saurabh Jayant Bhave, Deepak Kumar Mishra, Reena Nair

Introduction: Chronic myeloid leukaemia (CML) is characterised by good prognosis in chronic phase (CP). Progression to blast crisis (BC) is associated with poor outcomes. Mixed Phenotype Acute Leukemia (MPAL) in CML-BC is a very rare entity. Herein, we report one such case of CML with mixed phenotypic BC with compound TKI (Tyrosine Kinase Inhibitors) domain mutation, which posed great challenge in diagnosis as well treatment.

Result: Case report: 40-year-old male presented with fatigue, unexplained weight loss and hepatosplenomegaly. Blood investigations revealed leucocytosis (4,72,000/cu.mm), bone marrow (BM)was hypercellular with 3% blasts and significant shift to left. Fluorescent in situ hybridisation (FISH) for the BCR-ABL1 fusion was positive and reverse transcriptase polymerase chain reaction (RT-PCR) identified fusion transcript, e14a2, confirming diagnosis of CML in CP (EUTOS: high risk).

He was started on therapy, initially with Dasatinib, discontinued due to recurrent grade 3 cytopenia. Subsequently switched to Imatinib resulting in failure of response (No hematological remission at 3 months & RQ-PCR [BCR-ABL]:41.5%, TKD mutation: negative). He had prolonged cytopenia and RQ-PCR after 6 weeks: 65.6%, after dose increment of Imatinib. [Figure]. BM revealed 84% blasts. Immunophenotyping showed 2 subsets of blasts: 63% gated cells positive for CD34, CD10, HLADR, TdT, CD38 and CD56. And 24% gated cells positive for CD34, TdT, CD117, CD19(dim), CD10 (in a subset), CD13, CD33, HLA- DR, CD56 (subset), CD38. Thereby, suggesting Blasts with Ambiguous Lineage (no expression of lineage specific markers). Immunohistochemistry on biopsy showed blasts positive for CD34, TdT, CD10, PAX5, CD19 ($\sim$ 50%) and negative for cMPO and CD117. TKI domain mutation analysis showed compound mutation in exon 4 (G250E) & exon 8 (A424G, E459K) of the ABL1 gene (p210 transcript), which were either poorly sensitive to Imatinib or resistant to other 2nd & 3rd generation TKIs including Ponatinib.

Discussion: Based on Immunohistochemistry, he was labelled B lymphoid blast crisis and started on paediatric inspired (ICiCLe protocol) chemotherapy. However, he developed disseminated fungal infection and further chemotherapy was aborted.

Conclusions: Treatment of CML-BC is arduous, but the addition of diagnostic perplexity and compound TKI domain mutation adds to complexity and leads to poor outcomes despite optimal therapy.

Acute Leukemia-Laboratory

An Unusual Recurrent Translocation in an Usual Acute Leukaemia

Sujata Raychaudhuri, Shilpi More, Dipti Sidam, Garima Dhull, Tathagata Chatterjee

Introduction: AML M5 is associated with specific chromosomal translocations t(8; 16)(p11;p13) and those involving the MLL locus at 11q23. It presents with hyperleukocytosis, extra medullary involvement and coagulation abnormalities.

We present a case of a 16 yr old boy with complaints of weakness and fatigue.The TLC was 4500/mm^2 with reduced platelet count. Peripheral smears showed large blasts with round open chromatin having abundant cytoplasm and vacuolations with few showing Auer rods and Phi bodies.Neutrophils showed pelger- huet anamolies.

Bone marrow aspiration showed 45% blasts with promonocytes and monocytic maturation.

Cytochemistry revealed Myeloperoxidase (MPO) while IHC showed CD 34, CD 117 and CD 68 positivity.

Flow cytometry showed bright positivity for cMPO, CD 34, HLA-DR, CD33, CD 38, CD 117, CD 64 and CD 15 while CD 14 was moderate; heterogeneous positivity was noted for CD11c and Dim positivity for CD 13 with CD56 as the aberrant marker.

Diagnosis of AML M5 was rendered based on cytomorphology and flowcytometry.

Molecular studies and karyotyping revealed an unusual chromosomal translocation of AML1 ETO fusion gene which is a rarity in AML-M5. No other genetic abnormalities like NPM1, FLT-3, TKD, FLT3 ITD or C -KIT were detected.

t(8;21) forming AML1 ETO fusion protein are seen in M2, M4, M0 and M1 and very rarely in M5.It shows morphologically mature neutrophils and hence blast counts are usually below 20%.

AML1-ETO fusion protein shows higher frequency of CD34, HLA-DR, Tdt, CD 13, CD19 and CD56 and lower CD33 and myeloperoxidase in the blasts.CD56 is expressed in patients with KIT mutations.

AML with t (8, 21) have good prognosis except those with loss of Y chromosome which explained the relapse in the present case.

Aims & Objectives: Flow cytometry in AML M5 with RT PCR mutational analysis.

Materials & Methods: Peripheral smears, Bone marrow smears, flow cytometry and molecular karyotyping.

Result: t(8,21) in AML M5.

Conclusions: We report here possibly the first case from India of an unusual genetic translocation t (8, 21) in AML M5. Clinical features and morphology serves as the clue to the presence of t (8, 21) in AML M5.

Utility of Immunohistochemistry in Diagnosing Hematolymphoid Neoplasms in Necrotic and Poorly Fixed Tissues: A Series of Challenging Cases

Pranav Raghuram, Mithraa Devi,Subramanian Kalaivani,Gurusamy Dharma Saranya,Rakhee Kar,Debdatta Basu

Introduction: Immunohistochemistry and flowcytometry has been an integral part of diagnostic workup of hematolymphoid malignancies for several years. Flow cytometry has its own limitations when the cells are not viable or fresh and in conditions where the specimen is not having adequate cellularity. In such scenarios immunohistochemistry can be relied upon as the main modality in lineage

identification even if the morphology is poorly preserved owing to necrosis or poor fixation.
Aims & Objectives: To demonstrate the use of immunohistochemistry in poorly fixed or necrotic tissues in diagnosing hematolymphoid malignancies.
Materials & Methods: A total of 6 cases with poorly preserved morphology either due to myelonecrosis or due to poor tissue fixation were studied. 4 cases taken retrospectively from the institute archives along with 2 new cases. All cases except one were subjected to a primary panel including CD34,TdT,CD20,CD3,CD10, and MPO. Further markers were done when these markers were non-contributory for a conclusive diagnosis. One case was subjected only to a limited panel of markers as it was a follow up case of ALL.
Result: Out of the 6 cases, 5 cases were bone marrow aspiration/biopsy with myelonecrosis causing diagnostic difficulty and 1 case was that of a poorly fixed lymph node excision biopsy. All the cases were successfully categorised into the specific type and lineage of origin with the help of immunohistochemistry. 4 out of 6 were diagnosed as B-ALL in the marrow, 1 was a follow up case of B-ALL and the other one as T-LBL in the lymph node (which was poorly fixed). CD10 was one of the marker which gave consistently strong positive staining in all the ALL which presented as myelonecrosis at the time of diagnosis.
Conclusions: In cases where morphology doesn't provide much information owing to necrosis or poor fixation, immunohistochemistry might turn out to be the trump card in clinching the diagnosis. It is always worth a try with immunohistochemistry before concluding the case just as a necrotic or poorly fixed tissue and asking for a repeat biopsy.

HLA- DR & CD 34: Double Negative Acute Myeloid Leukemias

Preeti Tripathi, Venkatesan S, Rajan Kapoor, Kundan Mishra, Jaswinder Kaur Bhatia

Introduction: Dual negativity for CD 34 and HLA—DR antigen in leukemia panel is an important finding in clinical practice. This combination of this finding though seen classically in Acute promyelocytic leukemia (APL), is not specific to it, but can be seen in many other myeloid leukemias. in small percentage of cases no characteristic mutation/cytogenetic abnormalities (negative for t15;17 and negative for NPM/FLT3 ITD mutation) are found and sometimes show morphology overlapping with hypogranular APL. In such cases it would be prudent to rule out any atypical/unknown variants of APL.
Aims & Objectives: We analysed CD 34 and HLA—DR dual negativity AML cases presented to us in last 2.5 years and correlated with their molecular/cytogenetics findings. The objective was to describe their morphological/flow and cytogenetic profile and correlation with treatment response.
Materials & Methods: All consecutive cases of newly diagnosed acute myeloid leukemias who presented to our hematology OPD/ward between Jan 2020 and Apr 2022 were studied to look for CD 34 and HLA—DR dual negativity in flowcytometry profile. Cases in which complete information/cytogenetics/molecular profile were not available, were excluded. The clinical records were pulled out from patient database after ethical committee clearance.
Result: A total of 106 new acute myeloid leukemia cases were encountered during study period out of which 26 were found to be dual negative for CD34 and HLA DR Out of these 26 cases, 09 cases (34%) were diagnosed as APL (either on morphology/or confirmed with flowcytometry/Cytogenetics). Amongst rest 17 cases, 08 patients (30%) showed characteristic cup shaped morphology and on cytogenetics showed either NPM or FLT3 mutations. 02 cases showed monocytic profile on Flowcytometry but surprising showed HLA DR neg.. HLA DR negativity was observed in a relapse case of monoblastic AML which could be expression of antigen alteration post chemotherapy/disease evolution. 02 cases were diagnosed as Megakaryocytic leukemia and 01 as pure erythroleukemia. Rest 04 cases had neither any specific suggestive morphology or cytogenetic profile and were labelled as AML –NOS.
Conclusions: The non- APL myeloid leukemias which are dual negative are commonly found to be associated with monocytic cup shaped morphology, NPM or FLT 3 ITD mutation. However, in small percentage of cases no characteristic mutation/cytogenetic abnormalities. In such cases it would be prudent to rule out any atypical/unknown variants of APL.

Exploring NK Cells Immunotherapy in AML

Priya Nandlal Mahto, Chetan Dhamne, Syed Hasan, Prashant Tembhare

Introduction: Outcomes of relapse/refractory Acute Myeloid Leukemia (AML) are dismal even after High-dose chemotherapy and hematopoietic stem cell transplantation (HSCT). Studies from T cell-depleted transplants have shown that AML blasts are exquisitely sensitive to NK cells. Adoptive transfer of allogeneic NK-cells has shown activity against chemo-refractory AML. To increase the efficacy and persistence of adoptively transferred NK cells a memory-like phenotype of NK cells can be generated by incubating NK cells with cytokines including Interleukin-12, Interleukin-15, and Interleukin-18. Cytokine-induced memory-like (CIML) NK-cells have enhanced capacity to replicate over multiple generations in-vivo after infusion into the recipient. In addition to longer persistence, the CIML cell has enhanced cytotoxicity and increased capacity to secrete IFN-gamma.
Aims & Objectives: To demonstrate in-vitro the efficacy of cytokines-induced memory-like NK cells.
Materials & Methods: NK cells were purified using RosetteSep or CD56 + magnetic selection from human mononuclear cells. These cells are preactivated for 16 h using a cytokine cocktail (rhIL-12 + rhIL-15 + rhIL-18) vs control condition (rhIL-15). Cells were cultured in complete RPMI media and low-dose rhIL-15. On day 7, preactivated cells were restimulated for 6 h using a cytokine cocktail. Proliferation and cytotoxicity assays were carried out using CFSE by flow cytometry.
Result: We successfully purified NK cells using RosetteSep (purity $\geq$ 95.
Conclusions: The preliminary data suggest that CIML-NK cells have the potential to eliminate leukemic cells. In this study, we demonstrate that CIML NK-cells have enhanced cytotoxicity against AML blasts as compared to naïve NK cells. Having proven its efficacy, we plan to initiate a phase I/II clinical trial in r/r AML using CIML-NK cells. CIML NK cells can be used as a bridge to transplant in chemo-refractory AML.

The Frequency and Hematological Features of BCR::ABL1-Like Tyrosine Kinase Fusion Genes in B-Lineage Acute Lymphoblastic Leukemia

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Introduction: BCR::ABL1-like B-lineage acute lymphoblastic leukemia is characterized by the presence of translocation leading totyrosine kinase gene fusions or mutations leading to activation of various cellular pathways, namely ABL1-associated or JAK-STAT

pathways. The current report attempts to describe the frequency and hematological characteristics of B-ALL with tyrosine kinase gene fusions.

Aims & Objectives: To study the frequency and hematological features of BCR::ABL1-like tyrosine kinase fusion genes in B-ALL.

Materials & Methods: A retrospective analysis of B-ALL patients who underwent fluorescent in-situ hybridization using dual color-break-apart probes for ABL1, ABL2, PDGFRB, CRLF2, JAK2,FGFR1, EPOR, IGH,and deletion probe for P2RY8 on peripheral blood or bone marrow samples between 2018 and 2022 was performed.

Result: During our study period between January 2018and August 2022, a total of 276 patients who were negative for recurrent cytogenetic abnormalities(BCR::ABL1, TCF3::PBX1, KMT2Ar,andETV6::RUNX1) underwent FISH testing for above mentioned tyrosine kinase fusion genes.The age of patients ranged from 9 months–85 years (median:16 years; $\leq$ 12 years-85; $>$ 12 years-191).There were 195 males and 81females(2.4:1).The hemoglobin (g/dl), total leukocyte count($\times$ 106/L), and platelet count($\times$ 106/L) ranged from 2.4–14.4(median-7.4), 0.4–346 (median-12.2) and 1–857 (Median-28) respectively. Leukocytosis ($>$ 11 $\times$ 106/L)was present only in 38 (13.8%) patients.A tyrosine kinase gene fusion was identified in 33 patients,i.e.,12% of all cases or 28/191 adults (14.7%).These include P2RY8::CRLF2(n = 11; 4%), IGH::CRLF2(n = 7; 2.5%), PDGFRB-r(n = 6; 2%), IGH::EPOR (n = 4; 1.5%), JAK2-r (n = 3; 1.1%) andABL1-r (n = 2; 0.7%).The frequency of CD20 positivity was 64% (21/33) and 49%(120/243) respectively (p = 0.12), while expression of any myeloid marker was 54.5% (18/33) and 23.5% (57/243) respectively ($p < 0.001$). CD36 was positive in four cases, of which three had tyrosine kinase gene fusion.Follow-up data is available in 152 cases (55%). Induction failure or post-induction residual disease or relapse was seen in 83 cases (54.6%) and included 10/15 (66.7%) cases with tyrosine kinase gene fusion and 73/137 (53%) cases without tyrosine kinase gene fusion (p = 0.32).

Conclusions: BCR::ABL1-like tyrosine kinase gene fusion was identified in 12% of cases who underwent testing.The expression of myeloid markers, especially CD36, is significantly more frequent in these patients. Induction failure or post-induction residual disease or relapse is also more frequent in patients with tyrosine kinase gene fusions.

The Frequency of IGH Translocations in Adolescents and Adults with B-Lineage Acute Lymphoblastic Leukemia Lacking Recurrent Cytogenetic Abnormalities

Aishwarya Karthikeyan, Sreejesh Sreedharanunni, Sonia Rana, Parveen Bose, Shano Naseem, Man Updesh Singh Sachdeva, Arihant Jain, Alka Khadwal, Amita Trehan

Introduction: Translocations involving immunoglobulin heavy chain locus (IGH) are frequent in mature B cell neoplasms; however, they are also seen in patients with B-lineage acute lymphoblastic leukaemia (B-ALL). Here, we report the frequency and pathological features of B-ALL with IGH-rearrangement (IGH-r).

Aims & Objectives: To study the frequency and pathological features of B-ALL with IGH-rearrangement (IGH-r).

Materials & Methods: A retrospective analysis of adolescent and adult B-ALLs who were negative for recurrent cytogenetic abnormalities (RCA) [BCR::ABL1, ETV6::RUNX1, KMT2A-r and TCF3 r] and further underwent fluorescent in-situ hybridization/FISH using dual-color break-apart probes for ABL1, ABL2, PDGFRB, CRLF2, JAK2, FGFR1, EPOR, IGH, and deletion probe for P2RY8 on peripheral-blood or bone-marrow samples between 2018 and 2020 was performed.

Result: During the study period, a total of 278 patients ($>$ 12 years) were diagnosed of B-ALL. Among this, 118(42%) showed positivity for an RCA. Among remaining 160 cases, FISH for above mentioned panel could be performed in 144 cases. Twenty-eight cases (19.4%) were positive for IGH-r. The partners identified include CRLF2 and EPOR in three cases each. In remaining 22 (15.3%) cases, the partner was not known. Twenty one cases showed BCR::ABL1 associated chimeric gene fusions (CGF) including CRLF2 and EPOR rearrangements as mentioned above. Among B-others (after exclusion of RCA and BCR::ABL1 associated CGFs), IGH-r was seen in 17.7% (22/124) patients. On comparison of two groups among B-others, patients with IGH-r had significantly lower median hemoglobin (5.7 g/dl vs 7.3 g/dl; p = 0.15) and higher median total leukocytes count in $\times$ 10^6/L (34.6 vs. 9.9; p = 0.05). The expression of one or more myeloid markers was also more frequent in patients with IGH-r (36.5% vs 16.7%; p = 0.04). A follow-up was available in 60/124 cases. 60% (9/15) with IGH-r compared to 47% of remaining cases showed residual disease at the end of induction therapy (p = 0.37). The frequency of MRD positivity was 62% in patients with BCR::ABL1 like ALL and 38% (26/69) for other high-risk RCA (BCR::ABL1, KMT2A, iAMP21, and hypodiploidy).

Conclusions: IGH-r without an identifiable partner was detected in 15.3% of our cohort of adolescent and adult B-ALL who lacked RCA or BCR::ABL1 like CGF. They have significantly lower hemoglobin, higher leukocyte count and higher MRD positivity.

FISH Analysis to Identify Intragenic Deletions of IKZF1 GENE: A Rapid, Efficient, Cost Effective Strategy for Risk Stratified Therapy of Acute Lymphoblastic Leukemia

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Introduction: Deletions of the gene *IKZF1* that codes for the transcription factor Ikaros are seen in 70% of Ph positive and 30% of Ph negative B-cell precursor acute lymphoblastic leukemias (BCP-ALL) and are associated with a higher risk of treatment failure in both paediatric and adult ALLs. Majority of *IKZF1* deletions are intragenic with large chromosome 7 deletions accounting for 15% of *IKZF1* deleted patients. *IKZF1* deletions are routinely identified by performing Multiplex ligation-dependent probe amplification (MLPA) assay, fluorescent PCR and SNP array analysis. We developed a cytogenetic strategy based on fluorescent in situ hybridisation (FISH) using a commercially available probe that targets exons 4 to 7 of the *IKZF1* and identifies intragenic deletions. We compare the ability of FISH analysis in detecting *IKZF1* deletions, to the standard techniques.

Aims & Objectives: To validate FISH probe to identify intragenic deletions of the *IKZF1* gene in BCP- ALL.

Materials & Methods: FISH using IKZF1/CEN7 Dual Colour probe (ZytoVision GmbH, Bremerhaven, Germany) was performed on 13 BCP-ALL samples with known *IKZF1* deletion status on MLPA/fluorescent PCR/SNP array analysis. The orange fluorochrome labels exons 4–7 of the *IKZF1* gene spanning a 50 kb region and the green fluorochrome labels centromere region of chromosome 7 centromere (D7Z1). Cut-off of 7% was derived using control samples and beta inverse function.

Result: Of the 13 samples *IKZF1* deletions were present in 11 patients (entire gene deletion in 2, intragenic deletion in 9) on MLPA/fluorescent PCR/SNP array analysis. Seven patients had exon 4–7

deletions and two exon 4 to 8 deletions. FISH analysis was concordant in 9 of the 11 patients with *IKZF1* deletions and in both the patients without *IKZF1* deletions. Two patients with exon 4–7 deletions on fluorescent PCR were negative for deletions on FISH analysis suggesting the deletions were sub clonal.
Conclusions: FISH analysis was concordant with molecular assays to identify *IKZF1* deletions including intragenic deletions and is a rapid cost-effective alternative that can be easily incorporated in diagnostic laboratory settings.

FISH Strategy to Identify TCF3::HLF Fusion Positive B Cell Precursor Acute Lymphoblastic Leukaemia

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Introduction: The chromosomal translocation t(17;19) is a rare cytogenetic subtype of Precursor B cell Acute Lymphoblastic Leukemia (BCP-ALL) associated with dismal outcome. The resulting fusion gene on chromosome 19 encodes a chimeric transcription factor *TCF3::HLF*. The abnormality can be cryptic and with two types of transcripts at molecular level it is best identified by fluorescent in situ hybridization (FISH) analysis. We present two cases of this rare subtype with clinico-pathological correlation.
Aims & Objectives: To describe the clinico-pathological features of *TCF3::HLF* fusion positive ALLs.
Materials & Methods: The *TCF3* break-apart probe is included in our 4 probe FISH panel in BCP-ALLs. Patients positive for *TCF3* rearrangement on initial FISH analysis are investigated using a *TCF3* tricolour probe based on clinical features and cues from karyotyping. Karyotyping and FISH was carried out on bone marrow aspirate samples cultured overnight using standard cytogenetics protocols. FISH analysis was performed using *TCF3* break-apart (CytoTest Inc., USA) and tricolor fusion probe (Cytocell, Cambridge, UK)and GTG banded karyotype was reported as per the ISCN 2020 guidelines.
Result: Patient 1 (UPN1), a 7-year-old female presented with complaints of intermittent fever with a history of easy bruising and patient 2 (UPN2) a 11-year-old male presented with fever, bone pain and hypercalcemia. Baseline laboratory investigations, bone marrow aspirate examination and immunophenotype confirmed the diagnosis BCP-ALL with a negative CD34 expression in both the patients. In view of presence of DIC features (UPN1), hypercalcemia (UPN2) and absence of CD34 expression on blasts, interphase FISH analysis was performed initially using a *TCF3* break-apart probe to identify *TCF3* gene rearrangement followed by a tricolor fusion probe to confirm the presence of *TCF3::HLF* fusions. Karyotyping revealed a translocation between the long arm of chromosome 17 and the short arm of chromosome 19 in both the patients.
Conclusions: *TCF3::HLF* fusion positive patients present with DIC, hypercalcemia and low WBC counts and negative CD34 expression on immunophenotyping. FISH analysis using probes targeting *TCF3* gene is an efficient cost effective method to identify these patients.

Aberrant Antigenic Expression in Newly Diagnosed Acute Leukemias: A Study of 207 Cases

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Introduction: Leukemic cells express characteristic set of 'cluster of differentiation' (CD) markers, which forms the basis of the current WHO classification. Leukemia associated aberrant immunophenotype (LAIP) refers to expression of unusual CD markers by leukemic cells, which are not normally expressed by their respective lineage. The incidence of LAIP varies considerably in published literature, and its clinical implications, prognostic relevance and sensitivity to therapy are still debatable.
Aims & Objectives: To identify the immunophenotypic aberrancies in newly diagnosed cases of acute leukemias in our institute and to correlate the aberrant expression with prognostic markers.
Materials & Methods: This was an observational study, which included all newly diagnosed cases of acute leukemias on flow cytometry, over a period of four years. The demographic and clinical details, hemogram findings and peripheral smear and bone marrow findings were noted in each case. Aberrant immunophenotypic expressions were recorded whenever present and were correlated with available prognostic factors.
Result: The study included 207 newly diagnosed cases of acute leukemias. Of these 81 (39.1%) cases were acute myeloid leukemia (AML), 99 (47.8%) cases of B-acute lymphoblastic leukemia (B-ALL), and 27 (13.1%) cases of T-ALL. Immunophenotypic aberrancies were detected in 39.6% of acute leukemias. The highest incidence of aberrations was detected in AML (49.4.%), followed by T-ALL (40.7%) and B-ALL (29.3%). The most common LAIPs expressed in AML were CD7, CD56 and CD4. The commonest LAIPs detected in B-ALL were CD33, CD13, CD56; of which CD33 and CD56 expression correlated with poor prognostic factors like higher age and higher total leucocyte count respectively. In T-ALL, aberrant expression of CD13, CD56, CD33 and CD117 were commonly noted.
Conclusions: The results of this study have generated valuable baseline data on the incidence of LAIPs in this region. This information is vital because establishing LAIPs at the time of diagnosis is crucial for disease monitoring. Some LAIPs are associated with underlying cytogenetic abnormalities and hence impact the management and prognosis.

Diagnostic Dilemma Between Ph + Acute Megakaryoblastic Leukemia Versus Denovo Chronic Myeloid Leukemia in Megakaryoblastic Phase: A Rare and Interesting Case Report

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Introduction: Megakaryoblast crisis as the presenting manifestation of Chronic myeloid leukaemia is extremely rare and only few cases have been reported in literature. Similarly, incidence of Ph + acute megakaryoblastic leukaemia is extremely uncommon especially in adult age group. Differentiating denovo chronic myelogenous leukemia in blast phase (CML- BP) from BCR-ABL1-positive acute myeloid leukemia (AML) is a diagnostic challenge with therapeutic and prognostic consequences.
Case presentation: A 21-year-old female presented with complaints of fever, weight loss and abdominal swelling for one month. Clinical examined revealed massive hepatosplenomegaly. Lab investigations revealed anemia (Hb -6.6gm/dl), leukocytosis (20,000cells/uL) and adequate platelet count (324,000 cells/ul). Morphologic evaluation of the peripheral blood smear and bone marrow aspirate/biopsy revealed near complete replacement by blasts (intermediate to large size, moderate basophilic cytoplasm, open chromatin and cytoplasmic blebs) suggestive of megakaryoblasts along with occasional basophils. Flowcytometry confirmed megakaryoblastic lineage (CD34 + , cCD41 + , cCD61 + , cMPO-). Karyotyping, fluorescence in situ hybridization and RT-PCR analysis showed positive Philadelphia chromosome and p210 BCR::ABL1 (e14a2) fusion.

Aims & Objectives: To present a rare and interesting case report with a diagnostic dilemma between denovo megakaryoblast crisis in chronic myeloid leukemia and Ph + Acute megakaryoblastic leukemia.
Materials & Methods: 1. Peripheral blood and bone marrow examination—Diff quick stain
2. Flowcytometry—BD FACS CANTO II (Software used—BD FACS DIVA 8.0)
3. Fluorescence in situ hybridisation—Probes used: LSI BCR/ABL1 [dual fusion]
4. Karyotyping
5. RT-PCR for BCR-ABL1 fusion
Result: Diagnosis of chronic myeloid leukemia in megakaryoblast phase was favoured considering presence of splenomegaly, scattered basophils on peripheral smear, megakaryocytic hyperplasia/clustering on bone marrow biopsy, Ph positivity in 98% of cells and p210 transcript.
Conclusions: Chronic myeloid leukemia presenting in blast phase is rare only seen in 1–2% of CML patients especially in young adult age group. Differentiating de novo Ph positive AML from CML in blast crisis is a diagnostic challenge. Clinical history (chronic vs acute, age), examination (presence or absence of splenomegaly), peripheral blood/bone marrow morphology (basophilia/left shift/megakaryocytic morphology), cytogenetics (percentage of ph + cells) and molecular studies (presence of acute myeloid leukemia related mutations) may help in differentiating these two entities.

Assessment of Post-Induction Mrd Status in ETP/Near ETP Versus Non ETP ALL

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Introduction: The presence of minimal residual disease (MRD) is one of the strong predictors of disease outcome in various hematological malignancies including B-ALL and T-ALL, independent of pre-therapeutic risk factors. A variety of sensitive techniques like Multicolor FCM and PCR are in use for determining MRD. Studies suggest that MRD status in T-ALL maybe dependent on the diagnostic immunophenotype; specifically with regards to ETP/near ETP and non ETP phenotype.
Aims & Objectives: To assess the post-induction MRD status in patients with ETP/near-ETP and non-ETP immunophenotype in T-ALL.
Materials & Methods: Forty eight cases of T-ALL which were diagnosed by Multicolor Flowcytometry over a duration of 3 years were included in the study. The cases were defined as ETP/near ETP and non ETP ALL on the basis of expression of CD3, CD7, CD1a, CD5, CD8 and myeloid/stem cell markers. The post induction MRD status of cases with ETP/near ETP and non ETP phenotype were studied.
Result: The mean age was 20.8 years with a male predominance (M:F- 3.3:1). Fourteen patients (29%) presented with ETP-like phenotype out of which seven patients (14.5%) presented with ETP phenotype and another seven (14.5%) presented with near ETP phenotype. Out of the thirty four non-ETP cases, the majority (n = 22) were cortical phenotype (45.8%). At induction, four cases (57%) of the ETP subtype had positive MRD status, while one patient (14.3%) was found to have morphological persistence of blasts at the end of induction and the remaining achieved negative MRD status. All the patients of ETP phenotype with positive MRD showed similar pattern of LAIP expression at the time of MRD as at the time of diagnosis. Two patients with near ETP phenotype failed to achieve MRD at the end of induction. Three non-ETP cases (8.8%) had a positive MRD at the end of induction. The MRD positivity rates in ETP-like phenotype (ETP/near-ETP) were significantly higher than the non-ETP cases (P = 0.001).
Conclusions: ETP phenotype has a lower incidence than a non-ETP phenotype. ETP-like phenotype (ETP/near ETP) had a much higher rate of post-induction MRD positivity in comparison to non ETP phenotype. However, the study has to be validated on a larger cohort for validation of the results.

Hematological, Immunophenotypic, and Cytogenetic Features of Acute Promyelocytic Leukemia with PML::RARA Negativity on Reverse Transcriptase Polymerase Chain Reaction

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Introduction: Acute promyelocytic leukemia (APL) is characterized by the presence of t(15;17)(q24.1;q21.2)(PML::RARA) in 95% of cases and is detectable by reverse transcriptase PCR (RT-PCR). Remaining 5.
Aims & Objectives: To report hematological, immunophenotypic, and cytogenetic features of APLs with PML::RARA negativity on RT-PCR.
Materials & Methods: A retrospective analysis of patients with peripheral blood and bone marrow findings suggestive of APL, however negative for PML::RARA on RT-PCR but positive for RARA rearrangement by FISH using break-apart probes (Metasystems, Germany) was performed. In most of these patients, FISH was also done for PML::RARA and ZBTB16::RARA by dual color fusion probes (Cytotest Inc, USA, and Metasystems, Germany), and next generation sequencing (NGS) for gene fusions was performed in two cases.
Result: Fourteen cases satisfied our inclusion criteria over five years (January 2018 to August 2022). The age of patients ranged from 14–55 years (median:34 years). There were nine males and five females. Hemoglobin (g/dl), total leukocyte count (× 106/L), and platelet count (106/L) ranged from 3.5–11.7 (median-8), 0.6–39.9 (median-23.4) and 10–145 (Median-43) respectively. Anemia and thrombocytopenia were present in all cases, while leukocytosis was present in 10 (71%) patients. Peripheral blood and bone marrow abnormal promyelocyte count (%) ranged from 3–82 (median-49) and 22–83 (median-75). Granulocytic dysplasia was seen in two cases. All cases showed characteristic immunophenotypes (CD13pos, CD33pos, CD34neg, HLA-DRneg). On FISH testing, seven cases (50%) were positive for PML::RARA, and three cases (21.4%) for ZBTB16::RARA. Among remaining four cases, one case each showed positivity for STAT5B::RARA and IRF2BP2::RARA, respectively, by NGS. In two cases, the partner of RARA was unknown, but ZBTB16 was excluded by FISH testing.
Conclusions: Patients with APL diagnosed morphology but negative for PML::RARA on RT-PCR should undergo FISH testing to detect PML::RARA with alternate breakpoints and variant APLs. A combination of FISH using RARA break-apart probes and dual fusion

probes for PML::RARA and ZBTB16::RARA identifies most cases. Remaining patients require NGS for partner identification.

Myeloid Sarcoma of the Breast with Synchronous Early Precursor T-Acute Lymphoblastic Leukemia: A Rare Presentation

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Introduction: Myeloid sarcoma is a rare tumor which usually presents as an isolated extramedullaryhematopoietic tumor, either synchronously with an acute myeloid leukemia (AML) ormyeloproliferative neoplasm (MPN), and rarely arise de-novo. The most common sites involved are skin, lymph nodes, gastrointestinal tract, soft tissues, testis and bones. Rarely, it can involve central nervous system, oral mucosa, breast, genitourinary system and pleura.
Aim and objective We report a rare presentation of myeloid sarcoma of the breast with synchronous ETP-ALL.
Material and methods: IHC for CD45 and MPO were used to diagnose myeloid sarcoma. ETP-ALL was diagnosed with multiparametric flow cytometry and IHC for CD1a and CD8 IHC on trephine biopsy.
Results: A 24-year-old female presented with bilateral breast lumps. A routine hemogram revealed a total leucocyte count of 3.2 lakhs/mm3. The peripheral blood showing features suggestive of MPN favouring chronic myeloid leukemia (CML) in chronic phase (1%blast, and 2%basophils). However, FISH and RT-PCR for BCR-ABL was negative. The breast lump was biopsied and showed features of myeloid sarcoma(CD45, MPO positive on IHC). Within a month, she presented with 30% blasts in peripheral blood. She was treated with 3 + 7cytarabine and daunorubicin AML induction and high dose Ara-C consolidation(HIDAC). She achieved CR at the end of induction. At three months follow up, she presented with rapid enlargement of the breast lumps, bilaterall cervical lymphadenoptahy, hyperleukocytosis and > 80%blastsinperipheral blood. Flow cytometry proved it to be early precursor T-acute lymphoblastic leukemia (ETP-ALL).Then the patient subsequently treated with paediatric BFM protocol and planned for salvage chemotherapy with FLAG-IDA.The patient had refractory disease and did not responded to the treatment and died due to intracerebral hemorrhage.
Conclusion: ETP-ALL is a T-lymphoblastic leukaemia arising from bone marrow stem cells of T-lymphoblasts. This leukemia expresses one or more stem cell and myeloid associated markers but lacks expression of thymic T- cell markers (CD1a), mature T-cell markers and are MPO negative. The synchronous occurrence of ETP-ALL and myeloid sarcoma of the breast in our case possibly point to the promiscuity of leukemic stem cells and stem cells of ETP-ALL.

Flow Cytometry Diagnosis of Pediatric Leukemia's by Beckman Dx Flex Flow Cytometer: Study in a Tertiary Care Hospital and a Reference Laboratory

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Introduction: In pediatric age group leukemia's are the most common malignancies encountered and Flow cytometry Immunophenotyping is an important tool in the diagnosis of pediatric leukemia as categorizing them lineage wise helps in the specific treatment protocols which is prognostically significant for early treatment and response.
Aims & Objectives: To describe and classify the leukemia's in pediatric age group diagnosed by the flowcytometry Immunophenotyping.
Materials & Methods: In total 33 pediatric samples received for a period of six months with the suspicion of acute leukemia were subjected to flowcytometry Immunophenotyping. 29 Bone marrow samples and 4 peripheral blood samples analyzed by Beckman Dx Flex flow cytometer 12 color by comprehensive panel of antibodies according to consensus guidelines. Eight cases of reactive immunophenotype cases were excluded from the study.
Result: Totally 25 cases (Refer table-1) were diagnosed as acute leukemia's. 18/25 cases diagnosed as Precursor B acute lymphoblastic leukemia's (B-ALL). Out of the 19 cases of B-ALL one was CD10 negative B –ALL, one case had CD7 aberrant expression and one was diagnosed as Burkitt's leukemia. 3 /25 cases diagnosed as Precursor T acute lymphoblastic leukemia. Out of the three Two cases are near early T precursor acute lymphoblastic leukemia (NETPALL) and one case of early T precursor acute lymphoblastic leukemia (ETPALL).
Conclusions: This study highlights that in the diagnosis of pediatric patients, B -ALL is the most common pediatric leukemia followed by T cell ALL. Cases of myeloid leukemia's are reported but both the cases of AML reported within one year of age. Newer entities like ETPALL and near ETPALL were also reported.

Early T-Cell Precursor Acute Lymphoblastic Leukemia with Auer Rods: A Rare Finding with Review

Prapti Acharya, Gaurav Chhabra, Chinmayee Panigrahi, Somanath Padhi, Prabodha Kumar Das

Introduction: Early T cell Precursor—Acute Lymphoblastic Leukemia [ETP-ALL], is a hematolymphoid malignancy with the blasts demonstrating T cell lineage and a characteristic immunophenotypic pattern suggesting early T cell differentiation along with stem cell and myeloid antigen expression. The diagnosis of ETP-ALL from non-ETP ALL and mixed phenotype acute leukemia (MPAL) is often challenging due to its overlapping immunophenotypic picture with co- expression of myeloid antigens. Morphologically, the presence of Auer rods has been traditionally used as diagnostic of myeloid phenotype and has been very rarely seen in lymphoid blasts.
Aims & Objectives: We describe a rare case of ETP-ALL, which showed the presence of blast with lymphoblast like morphology, with presence of Auer rods in a few of them.
Materials & Methods: A 75-year-old female presented with complaint of atypical chest pain in cardiology. The physical examination was not significant and no organomegaly or lymphadenopathy was noted. The patient was clinically diagnosed as acute coronary syndrome and was advised PTCA. The further work up included CBC analysis that revealed leukocytosis with 40% blasts with occasional blasts showing Auer rods on peripheral smear and provisionally favoring a diagnosis of acute myeloid leukemia. Further, on flowcytometric immunophenotyping, the blasts were positive for CD34, cyCD3, CD7, CD13, CD117, HLA-DR, CD2, and negative for CD19, CD79a, cyMPO, sm CD3, CD14, CD64, CD33, CD1a, nuTdT, CD8, CD4 and CD5. Based on the morphological and immunophenotypic findings, a final diagnosis of ETP-ALL was given.
Result: This case describes an uncommon finding of the presence of Auer rods in the blasts of lymphoid origin. ETP-ALL and MPAL show the overlapping immunophenotypic markers with co- expression of T cells and myeloid antigens. This further becomes important as the management of both these entities is different. As per WHO the blasts in ETP-ALL are characteristically negative for MPO. In this case flow cytometry played an important role in making a correct diagnosis of ETP-ALL.
Conclusions: In clinical settings where the flowcytometry facilities are not available and diagnosis are based on morphology alone, it is

important, to be aware of such variations, that may result in misdiagnosis of these cases as acute myeloid leukemia.

Terminal Deoxynucleotidyl Transferase (TDT) Positive Acute Myeloid Leukemia

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Introduction: Terminal deoxynucleotidyl transferase (TdT) is expressed in nearly all cases of acute lymphoblastic lymphoma/leukemia (ALL) and in up to one-third of cases of acute myeloid leukemia (AML) with minimal differentiation. The prognostic significance of TdT positivity in AML is not clear and this requires further studies.
Aims & Objectives: To describe the clinico-morphological, immunophenotypic characteristics of a series of TdT positive AML cases and correlate the same with molecular characteristics with review of literature.
Materials & Methods: We describe the peripheral smear and bone marrow morphology, flow cytometric immunophenotypic and molecular characteristics of four AML subjects that showed TdT expression by immiunohistochemistry (IHC) and/or flowcytometry and reviewed the data on 61 cases of TdT positive AMLs published in last two years (2020–2021).
Result: TdT expression was noted among three males and one female (age range; 14 to 61 years). All had high blast count with presence of *undifferentiated myeloid blasts* (50 to 95% of marrow nucleated cells). These blasts had a small to intermediate size morphology with high nuclear to cytoplasmic ratio, scant sparsely granular cytoplasm without any Auer rods, mimicking ALL. On IHC, diffuse *moderate to strong* nuclear positivity for TdT correlated with CD34 and CD117 positivity and a weak to negative MPO expression. Molecular testing (n = 3/4) revealed deletion 7q in one, translocation t (6; 17) (q15;p13) and t (4; 8) (q31.3; q24.1) in one [AML with myelodysplasia related changes (AML-MRC)], and mutated FLT3 in one. One died, one (AML-MRC) is alive with relapse (follow up; 5 months), and the case with FLT3 mutation is in remission, where as the remainder was lost to follow up. Literature review on AMLs with intermediate risk cytogenetics (n = 48) revealed that TdT positivity (n = 12) was more likely to occur in elderly (median age; 73 years), and was associated with inferior *relapse free survival* than TdT negative subgroups (n = 36) (p = 0.002). In another retrospective review on 143 cases of AML, TdT [removed] n = 49) was linked to increased FLT3-ITD and NPM1 mutations. The median fluorescence intensity (MFI) of TdT was higher in FLT3-ITD subgroup than NPM1 subgroup, and had a poor overall survival compared to TdT negative subgroup (n = 94).
Conclusions: TdT positivity in AML may be a separate subgroup linked to specific underlying molecular abnormality that may affect the prognosis.

Aberrant Antigenic Expression in Acute Leukemia: Study from a Tertiary Care Center in West Bengal

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Introduction: Aberrant antigenic expression in acute leukaemia means an expression of antigens which is not normally associated with that specific lineage and does not fulfil the criteria for diagnosis of mixed phenotype acute leukemia (MPAL).
Aims & Objectives: This aberrant immunophenotype can be used for the assessment of measurable residual disease (MRD), sometimes may predict certain genetic events & prognosis of the disease. Certain markers may be of therapeutic target.
Materials & Methods: This is a cross-sectional study conducted in a tertiary care center in West Bengal from November 2021 to August 2022. Data about flow cytometry immunophenotyping (FCM IPT) from peripheral blood or bone marrow from all cases of acute leukemia attending the hematology OPD & admitted in ward within that period were collected to assess the type and frequency of aberrant antigen expression. All cases of acute leukemia confirmed by FCM IPT were enrolled.
Result: Total 279 cases of acute leukemia were diagnosed. Among them 115 acute myeloid leukemia (AML) (41.21%), 138 B- acute lymphoblastic leukemia (BALL) (49.46%) and 26 T- acute lymphoblastic leukemia (T ALL) (9.3%) were found. Aberrant antigenic expression was seen in 41 cases of AML (35.65%), 28 cases of BALL (20.28%) and 3 cases of T ALL (11.53%). Most common aberrant expression among AML was CD7(56%) followed by CD19 and among BALL was overexpression of CD58(89%). Among T ALL cases, CD79a, CD1a & CD13 were found in equal frequency (33.33%).
Conclusions: More aberrancy was found in cases of AML compared to BALL & T ALL. The future implication of this study is that aberrant marker can help in disease monitoring & some of them can be therapeutic targets. The limitation of this study is that correlation of aberrant markers with molecular or cytogenetic subtypes of acute leukemia has not been studied.

Simultaneous Presentation of RUNX1::RUNX1T1 Translocation Positive Acute Myeloid Leukemia and Multiple Myeloma. A Rare Occurrence

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Introduction: Acute myeloid leukemia (AML) has been seen and reported after treating multiple myeloma (MM). However simultaneous occurrence of AML and MM without prior exposure to chemotherapy is rare. Here we report a patient with simultaneous occurrence of AML with RUNX1::RUNX1T1 translocation and MM.
Aims & Objectives: –
Materials & Methods: –
Result: 56 year old male patient presented back ache with pain radiating to both legs. On evaluation, he was found to have a sacral mass, the biopsy of which showed a myeloid sarcoma. Peripheral blood showed normal hemoglobin (12.1 g/dl), total leukocyte count (6.4 × 106/L), and platelet count (280 × 106/L). Peripheral blood film examination showed the presence of myeloid blasts with Auer rods. Bone marrow examination and flow cytometry confirmed acute myeloid leukemia; however, with presence of 29% plasma cell. Flow cytometry confirmed clonal nature of plasma cells with CD38dim, CD138pos, CD45(dim), CD200pos, CD27dim, CD28dim, CD56pos, CD81dim, CD117pos, cytoplasmic lambda restricted immunophenotype. Reverse transcriptase PCR showed the presence of RUNX1::RUNX1T1 translocation and fluorescent in-situ hybridization of CD138 immunomagnetically sorted plasma cells showed deletion of 17p13 (TP53); however negative for FGFR3, MAF, MAFB, and CCND1 translocations. Serum protein electrophoresis (SPEP) showed an "M" band which on immunofixation electrophoresis was found to be IgG lambda. The biochemical investigation showed elevated LDH (315 IU/L) and beta-2-microglobulin (4.21 g/dl); however, it did not show hypercalcemia or renal failure. Imaging showed altered signal changes in sacral, lumbar vertebral bodies and pelvic bones. A diagnosis of AML with RUNX1::RUNX1 translocation with MM (revised international

staging system stage II) was made. The patient was given supportive treatment and finally succumbed to death.
Conclusions: Simultaneous presentation of two malignancies in the absence of previous therapy is rare and what predisposed him to such a rare phenomenon remains an enigma. While AML could have been quickly confirmed based on a peripheral blood film examination, the diagnosis of MM would have been missed in the absence of a bone marrow examination. The case thus highlights the importance of bone marrow examination in hematology practice.

T Regulatory Cells in Acute Leukemia and their Relation with Induction Outcome

Arunima Gupta, Preeti Tripathi, S Venkatesan, Rajan Kapoor, Kundan Mishra, Jasvinder Kaur Bhatia

Introduction: T regulatory cells are a subpopulation (5–10%) of CD4 + T cells that suppress the proliferation and function of immune cells through cell-to-cell contact. Therefore the enrichment of T regulatory cells at tumor sites is crucial for tumor cell proliferation.
Aims & Objectives: 1. To assess the percentage of 'T' regulatory cells in peripheral blood by flow cytometry prior to induction chemotherapy.
To find the association between percentage of 'T' regulatory cells and outcome to induction chemotherapy.
Materials & Methods: 55 cases of Acute Leukemia diagnosed and managed at Army Hospital Research & Referral and 10 healthy controls were assessed during our study. Acute leukemia cases in which the data on remission status was unavailable were excluded from the study.
Result: On flow cytometric analysis it was found that compared with those of healthy participants, the frequencies of CD4 + CD25 + TRegs in the peripheral blood of acute leukemia patients were significantly increased (1.5% [range: 0.50 to 4.3%] vs 0.58% [range: 0.40 to 1.05%], p = 0.0001). Our results were in concordance with previously conducted studies. Additionally, we also found that the frequencies of TRegs in the peripheral blood of cases who did not achieve remission were significantly increased (2.9% [range: 0.20 to 4.3%] vs 0.1.3% [range: 0.30 to 2.9%], p = 0.002).
Conclusions: We established that frequencies of Tregs is higher in acute leukemia cases when compared to healthy controls.We also found a negative correlation between percentage of Tregs and induction outcome. This study opens up a new possibility of using T regulatory cells as prognostic indicators of Acute Leukemia. It also suggests the possibility of using anti-Treg immunomodulators to improve the outcome of disease and response to chemotherapy.

Preferentially Expressed Antigen of MELANOMA (PRAME) Gene Expression in Acute Leukemia Patient

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Introduction: Acute leukaemia (AL) is a malignant haematological neoplasm associated with undifferentiated precursors clonal expansion, resulting in impaired haematopoiesis and bone marrow failure. Preferentially expressed antigen of melanoma (PRAME) gene regularly overexpressed in AL and other malignant diseases which are recognized by human leucocyte antigen (HLA-24) located in the human chromosome of 22q11 coded by 509 amino acids.
Aims & Objectives: To rule out the PRAME gene expression in AL patients and its correlation with clinical characteristics in the Indian population set up.
Materials & Methods: The transcript level of PRAME gene was detected in peripheral blood from 42 clinically diagnosed acute leukemia patients and 21 healthy individuals by Reverse transcriptase- quantitative polymerase chain reaction (RT-qPCR).
Result: A total of 42 samples collected, 29(69.4%) were males, and 13(30.95%) were females, with a mean and standard deviation for age were 39.07 ± 22.22 years. Of which AML were of 22(52.38%) cases, ALL were of 14(33.33%) cases and 6(14.2%) cases which included other forms of leukemia. PRAME gene expression was highly expressed in thirty-three 27(64.28%) AL patients compared to the least expression in healthy individuals. No Significant difference between the different forms of AL (p = 0.3203) was observed. Cytogenetic analysis of Normal Karyotype (NK), Abnormal Karyotype (Ab. K) and Culture Failure (CF) displayed statistical non-significance (p = 0.5801). Among cytogenetic abnormalities obtained no significant differences between the groups were observed (p = 0.8507). Chloride, potassium and absolute lymphocyte count (ALC) was found to be statistically significant with p = 0.0038**, p = 0.0358* and p = 0.0216* respectively between all other clinical characteristics. There was no correlation between the PRAME gene expression and clinical parameters.
Conclusions: PRAME gene expression in AL patients was highly expressed, comparable to studies reported globally with significant cytogenetic results. PRAME gene could be used as a potential diagnostic marker for monitoring malignancies, minimal residual disease, and targeted-based immunotherapy in AL if more studies come up with clinical trials.

Systematic Analysis of Fluorescence In Situ HYBRIDIZATION (FISH) Signal Patterns Using ETV6::RUNX1 Fusion Probe Risk Stratifies Almost 50% of Pediatric BCP-ALLs

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Introduction: Precursor B Cell Acute Lymphoblastic Leukaemia (BCP-ALL) is characterized by a constellation of chromosomal abnormalities that are prognostic and predictive of outcomes. The t(12;21)/*ETV6::RUNX1* fusion is seen in 15–20% of BCP ALLs and confers standard cytogenetic risk. The translocation is cryptic and is identified by FISH analysis using dual colour fusion probe. FISH analysis not only identifies *ETV6::RUNX1* fusions but also identifies iAMP21, *ETV6* deletion and is a screening tool to identify high hyperdiploidy (HeH), near triploidy (NT), tetraploidy, *ETV6* gene rearrangements and masked hypodiploidy. We describe the FISH patterns observed in 653 patients of BCP ALL using *ETV6::RUNX1* fusion probe.
Aims & Objectives: To evaluate the utility of FISH analysis using *ETV6::RUNX1* fusion probe in identifying different cytogenetic subtypes of BCP ALL.
Materials & Methods: Karyotyping using GTG banding and FISH using extra signal dual color probe for *ETV6::RUNX1* (Vysis/Abbott, IL, USA) probe, (ZytoVision GmbH, Bremerhaven, Germany) probe, (Metasytems GmbH, Altlussheim, Germany) probe was performed on 653 newly diagnosed paediatric BCP ALLs patients (2014–2021) as per standard protocols. Additional FISH analysis was carried out in a subset of cases.
Result: The median age was 4 years (9 months to 17 years) with a male preponderance (1.77). The *ETV6::RUNX1* fusion was seen in 122 patients (18.7%). The standard fusion pattern was present in 42 patients while 80 patients had atypical signal patterns. An additional *RUNX1* signal with deletion of one copy of *ETV6* gene was seen in 18 patients. *ETV6* gene deletion was present in 105 patients, 70 of these showing *ETV6::RUNX1* fusions. Gain of *RUNX1* as discrete signals (3 or more copies) suggesting HeH in 279 cases, NT in 7 cases and Tetraploidy in 8 cases respectively subsequently confirmed

by Karyotyping or additional FISH analysis. Variant *ETV6* gene rearrangements were identified in six patients and confirmed by FISH using *ETV6* breakapart probe. Five patients were diagnosed as iAMP21.

Conclusions: The frequency of t(12;21) in our study is less compared to western literature. Systematic analysis of signal patterns using the *ETV6::RUNX1* probe identifies multiple cytogenetic entities i.e. *ETV6::RUNX1* fusions, *ETV6* deletion, additional *RUNX1* signals, iAMP21, HeH and variant *ETV6* gene rearrangements. It risk stratifies almost 50% of paediatric BCP ALLs, thus being an efficient tool in resource constrained settings.

Role of CD58 and CD81 Expression in Discrimination of Hematogones from Lymphoblasts for Accurate Evaluation of Minimal/Measurable Residual Disease in Patients of Paediatric B-ALL

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Introduction: Minimal or measurable residual disease (MRD) is the presence of persistent populations of leukemic blasts after therapy at a level much lower than can be readily detected by conventional methods. It stands out as the most important independent prognostic factor for B cell acute lymphoblastic leukaemia (B-ALL), since it enables risk categorization and is highly predictive of relapse.

The discrimination of leukaemic lymphoblasts in follow-up of B-ALL by flow cytometry is often challenging due to the presence of hematogones. They resemble lymphoblasts morphologically and sometimes immunophenotypically. They increase in number after therapy. It has been demonstrated that the markers CD58 and CD81 can discriminate between lymphoblasts and hematogones.

Aims & Objectives: We aimed to evaluate the role of CD58 and CD81 in the assessment of MRD in paediatric B-ALL by evaluating their [removed]MFI and percentage positivity) on hematogones and lymphoblasts in cases of B-ALL at day + 35 of induction therapy.

Materials & Methods: 41 paediatric B-ALL cases who were on their day + 35 of induction therapy formed our study group. On day + 35 after induction therapy, bone marrow aspirate samples were collected in heparin vials for the patients.

For each sample, a 3 tube 5 colour panel using CD45, CD19, CD10, CD34, CD38, CD20, CD22, CD58 and CD81 were acquired on a Cytomics FC 500 (Beckman and Coulter). Data analysis was done using CXP Analysis software by gating on CD45dimCD19 + CD10 + (Blast gate) events for [removed]MFI and percentage positivity) of the said markers.

MRD was said to be positive when the persistent blast population comprised of atleast 0.01% of total mononuclear cells.

Result: 8 out of 41 patients were found to have a measurable residual disease of $> 0.01\%$.

Qualitative pattern expression of antigens showed overexpression of CD58 by the blasts when compared to hematogones, which was statistically significant ($p < 0 >$).

Conclusions: We demonstrate that CD58 and CD81 are important and valuable markers for MRD detection in B-ALL as they help to accurately distinguish between hematogones and lymphoblasts.

Pattern of Tumor Lysis Syndrome in Patients with Acute Lymphoblastic Leukemia

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Introduction: Prevention and treatment of tumor lysis syndrome (TLS) depends on immediate recognition of patients at risk. Therefore, we conducted this study to determine the frequency and risk factors of TLS in patients with acute lymphoblastic leukemia (ALL).

Aims & Objectives: The aim of the study was to observe the frequency of Tumor lysis syndrome in patients with Acute Lymphoblastic Leukemia.

Materials & Methods: This cross-sectional study was conducted at the department of Hematology in Bangabandhu Sheikh Mujib Medical University (BSMMU), over a period of 12 months following approval of this protocol. Total 50 patients admitted with ALL were included in this study after careful history taking, examination and appropriate investigations fulfilling inclusion and exclusion criteria, irrespective of their gender, race, ethnic group and age. Ethical issues were ensured properly. After briefing the aims and objectives and potential risk and benefits, written informed consent was taken from each subject. Interviews were done by investigator herself using separate case record form. After editing and encoding, data was analyzed by computer with the help of SPSS 24.

Result: The mean age of patients was 21.24 ± 13.83 (SD) years with majority aged less than 20 years (54%) and male gender (62%). Prevalence of TLS was found to be 26% (n = 13), wherein spontaneous onset (n = 8, 61.54%) and lab TLS (n = 9, 69.23%) was more frequent than therapy induced TLS (n = 5, 38.46%) and clinical TLS (n = 4, 30.77%). The most common biochemical changes occurred within 3 days before chemotherapy and 7 days after initiation of chemotherapy among TLS patients was hyperuricemia (69.23 and 76.92% respectivelyo and hyperkalaemia (61.54 and 69.23% respectively) with significant differences compared to non-TLS patients (p value $< 0 >$ 21.62% respectively, p value < 0 OR = 13.07, $>$

Conclusions: TLS was commonly found in patients with ALL, wherein spontaneous onset and lab TLS was more common than therapy induced TLS and clinical TLS. However, a large multicenter study is needed to corroborate these findings.

Flow Cytometric Analysis of CD64 Expression and CD45 Gating in Distinguishing Acute Myeloid Leukemia with Monocytic Differentiation (AML-MD) from Acute Promyelocytic Leukemia (APML)

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Introduction: Immunophenotyping by flow cytometry is a helpful ancillary tool for the diagnosis and subtyping of AML. It is generally recognised that AML with a monocytic component (AML M4 and AML M5) closely resembles APML. The relevance of CD64 as a sensitive and specific marker along with CD45 gating for differentiating AML M4 and AML M5 from other AML subtypes, primarily APML, is highlighted in this study.

Aims & Objectives: To identify role of expression of monocyte-associated antigens (CD64) along with CD45 expression in differentiating AML-MD from APML.

Materials & Methods: This was a retrospective study done in Hematology section, Department of Pathology, ABVIMS &Dr. Ram Manohar Lohia hospital, New Delhi. The study involved 33 newly diagnosed cases of AML. The cases were retrieved from our records. A standard panel of antibody markers including CD3, CD5, CD7, CD10, CD19, CD22, CD13, CD33, CD14, CD64, cMPO, CD34, HLA-DR, CD117, Tdt were analyzed by flow cytometric immunophenotyping in all the cases. In addition, CD45 vs side scatter (CD45/SSC) plots were also analyzed in all cases.

Result: A total of 33 cases of APML and AML-MD were analyzed by four color flow cytometry. 12/31 cases were diagnosed as APML, out of these 12 cases, 2/12 were microgranular variants and 1/12 was hypergranular variant. Remaining 19/31 cases were diagnosed as

AML-MD. 2 cases where definite diagnosis could not be provided, differentials of APML & AML-MD were suggested. CD64 expression was recorded in varying intensities in majority of cases of APML showing dim expression followed by dim to moderate expression, when compared to AML-MD where bright intensity of CD64 was noted in many cases, which helped in differentiating these cases along with CD45/SSC analysis. This revealed a higher percentage of monocytes in AML M4 and M5 when compared with APML and four different patterns—tear drop, cluster in blast region or monocytic region and cluster in blast region extending to monocytic region. These patterns further facilitated the differentiation of APML from AML-MD.

Conclusions: Bright CD64 expression strongly favours AML M5; however, it is difficult to distinguish all cases of AML M4 and M5 from APML by CD64 expression alone in cases with heterogeneous, dim, or moderate expression. CD64 expression combined with CD45/SSC analysis distinguishes such cases from APML.

GATA-3 IS a Highly Sensitive and Specific Marker for the T-Cell Lineage Assignment in the Immunophenotypic Classification of Acute Leukemia

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Introduction: GATA-binding protein3 (GATA3) is a transcription factor that regulates early T-cell differentiation. Current T-lineage definition in acute leukemia (AL) requires expression of surface or cytoplasmic CD3 (cyCD3) up to the level of mature T-lymphocytes. Routinely cyCD3 shows dim to negative expression, and T-cell-associated markers including CD7,CD5,CD4 and CD2 are not T-lineage-specific. Also in mixed-phenotypic AL cases it is challenging to confirm the diagnosis.

Aims & Objectives: There is a need of an additional marker for identification of T-lineage. We standardized GATA3 expression by multicolor flow cytometry(MFC) and evaluated its utility in T-lineage assignment.

Materials & Methods: Anti-GATA3(PECF-594, clone-L50-823) antibody was standardized and studied in the leukemic blasts using MFC. Intracellular staining was assessed using four different permeabilization reagents:FACS Lyse(BD Biosciences), Fix-&-Perm(Invitrogen), Foxp3-fixation-kit(eBiosciences) and True-nuclear transcription-factor staining buffer-set (Biolegend). Expression level of GATA3 was determined as normalized mean fluorescent intensity(nMFI) against internal negative control.

Result: Of the four reagents studied, FACS Lyse (BD Biosciences) was found to be the best permeabilization reagent for GATA3 expression. We studied 138 T-ALL, 32 AML, 18 MPAL, and 20 B-ALL patients. Mature B-cells and T-cells were taken as negative and positive controls with a median of nMFI of 0.52 (range 0.0 -8.95) and 1.32(0.0–6.73), respectively. Taking a cut-off of 2% positivity defined using ROC analysis, 138/138(100%) T-ALL and 13/18(72.2%) MPAL(15 T/myeloid and 3 B/T lymphoid), were positive and 20/20(100%) of B-ALL cases were negative for GATA3 expression. We also observed that 4/32(12.5%) cases of AML were also positive for GATA3 and had CD7 co-expression. Median(range) of nMFI of GATA3 in T-ALL, AML, MPAL, NK ALL, and B-ALL patients were 3.50(0.0–9.47), 0.17(0.00–6.32), 1.56(0.00–9.38), 5.93(0.00–5.93), and 0.00(0.00–0.10) respectively. We adapted a cut-off of 5%(instead of 2%) to define GATA3 positivity. The sensitivity and specificity of GATA3-expression for T-cell lineage using a cut-off of 5% positivity were 96.4 and 100% respectively.

Conclusions: We first-time standardized flow cytometric assessment of GATA3. FACS Lyse(BD-Biosciences) was the best permeabilization reagent. We conclude GATA-3 is a sensitive and specific marker for the diagnosis of AL with T-cell differentiation and its expression can provide additional evidence in the assignment of T-lineage in difficult cases of AL with weak cytoplasmic-CD3 expression.

Expression Pattern of CD244, a Novel SLAM Protein and its Clinical Utility in the Diagnosis of Acute Leukemia

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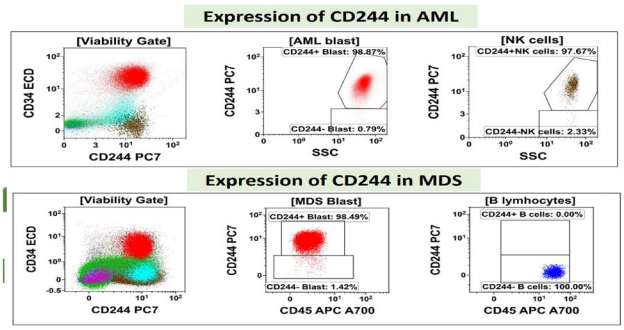
Introduction: CD244, a member of the signaling lymphocyte-activation molecule (SLAM), expressed on NK-cells, subpopulation of T-cells, monocytes, and basophils. CD244 has a vital role in maintenance of stemness of leukemia-initiating cells. There is a lack of studies evaluating its expression in acute leukemias (AL).

Aims & Objectives: With the advent of immunotherapy, we aimed to assess expression of CD244 in ALs and evaluate its role as a therapeutic target.

Materials & Methods: We studied expression pattern of CD244 (PECy7, C1.7) using a 10–13 color panel. The study included 54 B-ALL, 49 T-ALL, 92 AML, 20 MDS, and 7 uninvolved bone marrow (BM) controls. Post-acquisition analysis was performed using Kaluza software (v2). A cut-off of $\geq$ 10% used for defining, CD244-positive leukemic-blasts. Statistical evaluations performed using MedCalc (v14.8.1).

Result: CD244 was positive in 5/54 (9.26%) B-ALL, 24/49 (48.98%) T-ALL, 89/92 (96.74%) AML, 20/20 (100%) MDS cases. Evaluation of controls showed CD244 expression on monocytes, NK and T-cells. The median nMFI of CD244 in monocytes 6.46 (1.08–8.60), NK-cells 5.63 (0.11–9.31), T-cells 2.69 (0.30–7.78). Median nMFI of CD244 in leukemic-blasts of AML 8.96 (0.23–48.42), T-ALL 0.41 (0.00–9.96), B-ALL 0.03 (0.00–5.74). The median nMFI of CD244 in MDS-blasts 8.33 (1.35–9.71). CD244 expression was assessed in granulocytes, monocytes from AML, MDS. The median nMFI of CD244 in granulocytes and monocytes from AML was 3.00 (0.00–9.46), 4.00 (0.23–10.00) respectively and 2.00 (0.27–6.24), 10.00 (3.69–10.55) from MDS. CD244 expression distinguished AML-blasts from granulocytes ($p < 0.0001$), monocytes ($p < 0.0001$), and MDS-blasts from granulocytes ($p < 0.0001$), monocytes ($p = 0.0002$). In T-ALL, CD244 expressed on NK-cells, distinguished CD244-negative T-blasts from NK-cells ($p < 0.0001$). The median of CD244 in NK-cells 8.23 (0.04–9.99) and T-blasts 8.230.41 (0.00–9.96).

Conclusions: Current study is the first to evaluate expression of CD244 in AL. CD244, widely expressed in abnormal myeloid cells. In T-ALL, it helps identifying NK-cells from T-blasts. We highlight role of CD244 as potential marker for MRD-monitoring in AML where blasts can be distinguished from granulocytes/monocytes and T-ALL where T-blasts can be distinguished from NK-cells. The expression of CD244 on abnormal myeloid population indicates its potential as therapeutic target, subject to validation.



Flowcytomeric Immunophenotyping of Mixed-Phenotype Acute Leukaemia: A Report of Three Cases from A Tertiary Care Centre

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Introduction: Mixed-phenotype acute leukaemia (MPAL) is a rare neoplasm constituting 1–3% of all acute leukaemia and characterized by cells that express lineage-specific lymphoid and/or myeloid markers, thus showing a B/T lymphoid, B/myeloid, T/myeloid, or a trilineage B/T/myeloid phenotype.
Aims & Objectives: This case series attempts to identify cases of MPAL and study their clinical and haematological profile in a tertiary care referral centre in north India.
Materials & Methods: Ethylenediaminetetraacetic acid (EDTA)-anticoagulated peripheral blood samples of patients diagnosed with acute leukaemia (AL) on the basis of morphology over a period of 5 years were used for immunophenotyping. A comprehensive panel of fluorochrome-labelled monoclonal antibodies targeting myeloid, B-cell, T-cell and immaturity markers was applied. The patients were diagnosed with MPAL based on the World Health Organization 2017 classification and their clinical, haematological and flowcytometric immunophenotyping characteristics were studied.
Result: There were 3 (1.5%) patients with MPAL of the total 205 cases of AL. Case 1: A 2-year-old male child presented with fever and hepatosplenomegaly. Peripheral smear showed the presence of 72% blasts with lymphoid-type morphology. On flow cytometric immunophenotyping, the case was diagnosed as B/T lymphoid MPAL. Case 2: 21-year-old female presented with fever and hepatosplenomegaly. The blasts constituted 80% of total leucocytes in the peripheral blood and morphologically were of lymphoid type. Flow cytometric immunophenotyping revealed trilineage marker expression and the case was diagnosed as B/T/myeloid MPAL. Case 3: An 18-year-old male presented with acute cholangitis, parotid region swelling, and 7th nerve palsy. Blasts accounted for 47% of all leucocytes on peripheral smear and had the myeloid-type morphology. Flow cytometric immunophenotyping findings were of B/Myeloid MPAL. There was no specific morphology of blasts that could predict MPAL.
Conclusions: The cases highlight the varied presentations and phenotypes of MPAL. Identification mainly rests on flow cytometric immunophenotyping with a comprehensive primary panel of monoclonal antibodies. The prognosis of the disease is, in general, dismal, and there is at present no unified treatment for this special type of leukaemia.

BCR::ABL1 Fusion with Isochromosome 9 Along with A Ring Chromosome X in a Newly Diagnosed Case of B Cell All

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Introduction: B-cell acute lymphoblastic leukemia (B-ALL) is a disease found mainly in children and in young adults. It represents ~ 25% of cancer diagnoses among children younger than 15 years of age.
Aims & Objectives: To present an unusual case of newly diagnosed B cell ALL showing rare chromosomal abnormalities.
Materials & Methods: A 2-year-old female patient presented with high grade fever one month back and cough for two months having occasional abdominal pain. On examination she had rashes all over the body and splenomegaly. Haematological parameters showed Hb: 6.5 g/dL, WBC: 89 × 103/µl, Platelet: 89 × 103/µl and Blast: 90%. Flow cytometry from peripheral blood confirmed pre B cell ALL.
Conventional cytogenetics from unstimulated cell cultures (direct, overnight, 24 h and 48 h) followed by GTG banding was done. Sequential fluorescence in-situ hybridization (FISH) on G-banded metaphases using ZytoLight CEN X/Y Dual Color Probe was performed to confirm the presence of ring involvement on chromosome X.
Result: Chromosome analysis revealed isochromosome of long arm of chromosome 9 and translocation between chromosome 9 and 22 along with ring chromosome X having a karyotype 46,XX,i(9)(q10),t(9;22)(q34;q11.2)[2]/46,idem,-X, r(X)(p22.3q28)[12]/46,XX[2]. Molecular studies for ALL multiplex panel by PCR showed detection of BCR-ABL1 minor gene, and showed negative status for TCF3::PBX1 t(1;19)(q23;p13), KMT2A::AFF1 t(4;11)(q21;q23), KMT2A::MLLT3 t(9;11)(p21;q23), BCR::ABL1 (Major) t(9;22)(q34;q11), BCR::ABL1 (Micro) t(9;22)(q34;q11), KMT2A::MLLT1 t(11;19)(q23;p13.3), ETV6::-RUNX1 t(12;21)(p13;q22). Next generation sequencing revealed pathogenic BCR-ABL1.B1A2 mutation. The patient was given standard UKALL 2011 protocol for childhood ALL initially, and as patient was Ph positive B-cell ALL, further treatment was given according to EsPh ALL 2017¬. The patient is currently in remission with MRD negative status.
Conclusions: Cytogenetic studies proved to be an efficient technique in detecting the prevalence of rare, high prognostically significant structural aberrations. Nonrandom chromosomal aberrations have important prognostic significance in ALL. The occurrence of i(9)(q10) in newly diagnosed ALL is uncommon (0.6%–1%) and the presence of Ph chromosome along with ring X is rarest of rare event. The clinical significance of these findings are uncertain and needs to be explored. It has been reported that i(9)(q10) is usually associated with the late-stage disease also the ring chromosome found to be coincided with evolution from stable to the aggressive phase and hence, finding of an acquired ring chromosome is associated with unfavourable prognosis.

Correlation of Day8 Peripheral Blood Blast Count and Treatment Response in Cases of Paediatric Acute Lymphoblastic Leukaemia in Western Rajasthan

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Introduction: Acute lymphoblastic leukaemia (ALL) are the most common neoplasms of paediatric age group composed of precursor B or T cells. The classification requires incorporation of clinical details, morphology, cytochemistry, immunophenotyping and molecular genetic analysis. The survival rate of ALL depends on the age of the patient and the response to chemotherapy. Response to treatment is a strong outcome predictor for childhood ALL. This response can be assessed by blast count in peripheral blood on day8 of prednisolone therapy followed by bone marrow evaluation for measurable residual blasts at day 33.

Aims & Objectives: To evaluate the correlation between day8 blast count in peripheral blood and response of induction chemotherapy (Assessed by bone marrow morphology and measurable residual disease (MRD) status on day33).
Materials & Methods: The present ambispective study was conducted in Department of Pathology & Lab Medicine and Department of Paediatrics of AIIMS, Jodhpur. After obtaining approval from Institute's ethics committee, all paediatric ALL cases were included in the study. Cases were diagnosed based on morphology, flow cytometric immunophenotyping and molecular genetic findings. Day8 peripheral blood blast count were obtained. Bone marrow examination and MRD analysis were done at the end of induction. All clinical and laboratory data were entered in Microsoft excel sheet and analysed.
Result: A total of ten cases were studied. Of these, three were females and rest were male and their age ranged between two to nine years. Three cases showed $\geq$ 5% blasts in day8 peripheral blood. Of these, two cases showed MRD positivity (More than 0.01% on flow cytometry) on Day33 bone marrow and one case had MRD negative status. Other seven cases had shown peripheral blood blasts $<$ 5% and they were found to be in morphological remission as well as negative on MRD analysis by flow cytometry at end of induction bone marrow.
Conclusions: Early steroid response in paediatric ALL treatment is an independent prognostic factor in the prediction of patient outcome which can help in optimizing chemotherapy protocol.

Detection of FLT3-Internal Tandem Duplication in Acute Myeloid Leukaemia (AML): A Comparative Study of Targeted NGS To Standard Fragment Length (FA) Analysis

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Introduction: AMLis the malignancy of the myeloid lineage of hematopoietic stem cell. The FLT3 gene influences myeloid differentiation. FLT3 ITDs are present in approximately 25% of AML patient. It exhibits poor prognosis and has therapeutical implications (US FDA approved FLT3 inhibitors midostaurin). Traditionally, FLT3-ITD has been tested by FA method. However, NGS has been increasingly used. It provides comprehensive mutational profiling of clinical markers simultaneously including FLT3-ITD with a single method.
Aims & Objectives: We investigated the comparative analysis of FLT3-ITD using both FA and NGS method.
Materials & Methods: Retrospective study was carried out with 181 AML cases from 2019 to 2022. FA was executed as per literature on Genetic Analyzer 3500. NGS was performed using Oncomine™ myeloid assay on ION Gene Studio S5. In FA, AR was calculated from the fluorescence intensity ratio of the FLT3-ITD versus wild-type peaks obtained from capillary electrophoresis. In NGS it was calculated from the ratio of the number of sequencing reads showing FLT3-ITD versus wild-type sequences.
Result: Among 181 AML cases, FLT3-ITD was observed in 33 cases with variable sizes (14–166 bp). The frequency of FLT-ITD variant is 18.23%(N = 33/181). Both methods were performed in 143 cases. A concordance of 93.10% (n = 27/29) was seen in FLT3-ITD detection and high/low AR stratification by dual methods. However, for 2 cases, FLT3-ITD was detected byFA but it was not confirmed on the NGS assay, possibly due to a very low AR—0.006 and 0.05 (detected on FA) respectively or perhaps during NGS analysis, potentially informative reads flagged with sequence quality below set thresholds may have been discarded. In contrast, FLT3-ITD was detected by NGS for 4 cases but it was not tested by FA. Mean TAT of FA is 5 days and that of NGS is 15 days.
Conclusions: Majority of the FLT3-ITD positive cases exhibited concordance by dual methods. FA exhibits higher sensitivity for detecting lower AR and larger insert size duplication. Mean TAT of FA is lesser and it is a cost effective method to detect single variant when compared to NGS, though NGS provides comprehensive data of multiple genes simultaneously in a single assay. We perform both the methods to ensure timely risk stratification and commencement of FLT3-ITD inhibitor therapy in AML patients.

Aberrant Immunophenotypes with Special Reference to two Novel Markers: CD 123 & CD 25 in Acute Leukemia—A Study In Eastern India

Prannoy Das, Surabhi, Ruchi Sinha, Tarun Kumar, Punam Prasad Bhadani, Shreekant Bharti, Basab Bagchi

Introduction: Aberrant antigen expression helps to distinguish the neoplastic population from its non-neoplastic counterparts and detecting minimal residual disease. Flow cytometric immunophenotyping is crucial for identifying abnormal phenotypes. The prevalence of aberrant phenotypes varies widely among independent research, and its association with prognostic variables are still up for debate.
Aims & Objectives: To identify the prevalence of aberrant immunophenotypes in de novo acute leukemia (AL) and to evaluate their association with the clinical and hematological features.
Materials & Methods: A hospital-based cross-sectional study on flow cytometric immunophenotypic spectrum of 64 de novo AL patients (n = 26 AML, n = 33 B ALL, n = 5 T ALL) in the Department of Pathology, AIIMS, Patna. The study duration was from May 2020 to December 2021.
Results: CD7 (23.1%, n = 6) was commonest aberrant lymphoid antigen in AML, followed by CD4, CD8 and CD10. CD7pos AML showed an association with total platelet count (p = 0.012). CD33 (30.3%, n = 10) was most common aberrant myeloid antigen in B ALL, followed by CD13 and CD117. CD56 showed positivity in 19.2% and 27.3% cases of AML and B ALL cases, respectively. Among novel markers, CD123 was frequently expressed in all the subtypes of AL. Statistically significant association was found between CD25pos AML and leucocytosis, CD19neg AML with absence of expression of CD123; CD34pos and CD33pos B ALL with CD123 positivity status and CD13neg B ALL with absence of expression of CD25.
Conclusion: To conclude, the current study could not find any association between expression of aberrant lymphoid/myeloid antigen in AL and adverse clinical and hematological parameters. However, CD123 and CD25 expression may be employed as predictive/prognostic markers in AL, further studies with a larger sample size is necessary.

Flowcytometry Immunophenotypic Expression of APML: A Single Tertiary Center Study

Ankita Agrawal, Raka Hota, Rajesh Kumar Bhola, Sarita Pradhan, Ripunjaya Mohanty, Priyanka Samal, Debahuti Mohapatra

Introduction: Acute promyelocytic leukemia (APML) is a subtype of acute myeloid leukemia (AML) with distinct morphologic, biologic & clinical features including the presence of abnormal promyelocyte with bilobed nuclei and frequent Auer rods. Most patients exhibit a diagnostic t(15;17) (q22; q21) balanced translocation, resulting in a fusion transcript joining the promyelocyte (PML) and retinoic acid receptor α(RARA) genes. APML accounts for 5–8% of AML and is an emergency due to the high risk of developing life-threatening

coagulopathy if not treated promptly. Multiparameter flow cytometry has an established role in defining blast lineage, which provides a rapid diagnostic tool in case of APML when morphologic evaluation is suspected and later confirmed by PML-RARA transcription.
Aims & Objectives: To study the immunophenotypic changes of acute promyelocytic leukemia(APML) in patients who are clinically and morphologically suspected to provide a prompt diagnosis.
Materials & Methods: This is a retrospective study carried out in the Department of Hematology, IMS & SUM Hospital, Bhubaneswar from July 2020 to July 2022. The cytogenetics/molecular data with t(15,17) PML-RARA fusion transcription positive patients were retrospectively correlated with Flowcytometric immunophenotyping & clinical parameters. The cases were analyzed using Beckman Coulter DxFlex 3 Laser 13 color instrument as per the acute leukemia panel.
Result: A total of 26 cases of APML with t(15;17)/PML-RARA confirmed by conventional cytogenetics and/or FISH studies were analyzed for FC immunophenotyping. The age of patients ranged from 27 to 58 years (average 40.66). There were 12 men and 14 women. 15cases (57.6%) were characterized by high SSC, 2 cases (7.6%) had low SSC and rest showing moderate scatter, 13 cases were negative for CD34 and HLA-DR and 6 were positive both for CD34 and HLA-DR. 11 cases were showing CD117 bright, 8 were CD117 dim expression. 26 cases expressed CD13 bright positive, 14 were having homogenous bright CD33 expression. CD64 was negative in 6 cases.26 cases were negative for CD14, CD11c, CD4, CD36. CD56 was expressed in 1 case.
Conclusions: Flowcytometry immunophenotypic analysis can facilitate prompt diagnosis of APML, which helps in early therapy to overcome the fatality.

Hypoplatic AML in an Adolescent Male: A Rare Case Report

M Abinaya, Ruchi Sinha, Surabhi, Abhirami, Greeshma Gopinath

Introduction: Hypoplastic acute myeloid leukemia(AML) is a rare variant of AML, accounting for 5%-7% of all cases of AML. It usually occurs in elderly patients and is extremely rare in childhood and young age. It usually has an indolent course with relatively low tumour burden than usual AML. However, it has early mortality during chemotherapy.
Aims & Objectives: To describe an unusual occurrence of hypoplastic acute myeloid leukemia in young age.
Materials & Methods: Case Presentation: 16-year-old male presented with high grade fever, easy fatiguability and weakness for 1 month. On physical examination, he had pallor but no organomegaly or lymphadenopathies. Complete blood count (CBC) showed pancytopenia with flagging for atypical cells. Peripheral blood smear (PBS)confirmed the presence of 2% atypical cells. He was started on vitamin B.12 supplementation as his serum levels were low. Workup for infections were negative. Intravenous antibiotics was initiated in view of febrile neutropenia. No improvement noted clinically as well as hematologically. Bone marrow aspiration and biopsy studies showed hypocellular marrow(< 40 >
Result: Discussion: Acute leukemia presenting with hypocellularity has been known to occur rarely. Usually, hypocellular acute lymphoblastic leukemias occur in children. Hypoplastic AML has been rarely reported in paediatric and young patients. It needs to be differentiated from aplastic anemia and hypoplastic MDS. Hypoplastic AML is defined as hypocellular marrow with 20% or more blasts in bone marrow which are MPO positive. Recently, the beneficial effects of hematopoietic growth factors have been reported in the treatment of hypoplastic AML and also observed that chemotherapy may be necessary to maintain remission after treatment with hematopoietic growth factors.
Conclusions: Hypoplastic AML in young patients is rare, patients tend to have more profound cytopenia and its diagnosis is challenging for both pathologists and clinician.

ETP-ALL and Near ETP-ALL -Experience in a Single Tertiary Care Centre in Eastern India

Paramita Mukherjee, Sarita Pradhan, Rajesh Kumar Bhola, Priyanka Samal, Ripunjay Mohanty, Raka Hota, Gayatri Behera, Debahuti Mohapatra

Introduction: Early T-cell precursor acute lymphoblastic leukaemia (ETP-ALL) is a subtype of T-ALL recently added in the World Health Organization (WHO) in 2016 and is characterised by a distinctive immunophenotype. It accounts for 10–13% of childhood T-ALL and 5–10% of adult T-ALL. It carries a poor prognosis compared to other T-ALL.
Aims & Objectives: To characterise the clinico-pathological features and flow cytometry findings of ETP-ALL and Near ETP-ALL diagnosed in our centre.
Materials & Methods: All cases diagnosed as T-ALL were retrieved from July 2017 to June 2022 from database and their flowcytometry findings were analysed. The cases were categorised into ETP-ALL and Near ETP-ALL using the WHO 2016 criteria based on expression of the following immunophenotypic markers- CD1a, CD2, CD3 (surface vs cytoplasmic), CD4, CD5, CD7, CD8, CD13, CD19, CD33, CD34, CD117, HLA-DR, TdT, and myeloperoxidase. The ETP-ALL/LBL immunophenotype was defined as follows: (1) absent (< 5% positive cells) CD1a and CD8 expression, (2) absent or dim (< 75% positive cells) CD5 expression, and (3) [removed] > 25% positive cells) of 1 or more myeloid (CD11b, CD13, CD33, CD117) or stem cell (CD34, HLA-DR) markers. The cases with incomplete flow cytometry data were excluded from the study.
Result: Flowcytometry analysis of 62 cases of T-ALL were evaluated, out of which 9 cases (14.52%) were diagnosed as ETP-ALL and 4 cases (6.45%) as Near ETP-ALL. 8 out of 9 cases of ETP-ALL were above 18 years and 1 below 18 years. 2 out of 4 cases of Near ETP-ALL were above 18 years age and 2 out of 4 below 18 years. The most common immature marker was CD 34. The most common aberrant marker was CD 33. The MFI (The Mean Fluorescent Intensity) of CD 5 expression in T lymphocytes and blasts were compared in ETP-ALL and Near ETP-ALL.
Conclusions: ETP-ALL is a high risk subtype of T-ALL diagnosed through flowcytometry. The diagnosis is of paramount importance because of overlapping features between ETP-ALL with other non ETP-ALLs and T-lymphoid/Myeloid Mixed Phenotype Acute Leukaemia(T/M-MPAL).

Acute Megakaryoblasticleukemia: A Short Case Series from A Cancer Institute In Eastern India

Meera Mathayoth, Asish Rath, Mayur Parihar, Sushant Vinarkar, Sambunath Banerjee, Subhojit Brahma, Munmun Banerjee, Niharendu Gara, Reghu K S, Saurabh Bhave, Jeevan kumar, Reena Nair, Arijit Nag, Deepak Kumar Mishra

Introduction: Acute megakaryoblasticleukemia (AMKL) is a rare subtype of acute myeloid leukemia (AML) constituting 1% of adult and and 4%-15% of paediatric AMLs. We present clinical, haematological, flow cytometric immunophenotyping (FCM-IPT) and cytogenetic profile of 14 cases of AMKL.
Aims & Objectives: To study clinico-haematolgical, immunophenotype and cytogenetic features of patients diagnosed as AMKL.
Materials & Methods: All AMKL patients diagnosed at our institute from 2012–2022 were included. The 5-tube 8-color acute myeloid

leukemia panel was used for immunophenotyping. Cytogenetic evaluation was done as per standard protocols.

Results: The fourteen AMKL patients in our study included both children (71%) and adults (29%)with female preponderance (M/F = 1:1.3). Fever (92%) was the major presenting complaint. Only one patient was a known case of Down syndrome. Hepato-splenomegaly was seen in 44% of patients.

Peripheral smear and bone marrow aspirate/biopsy revealed MPO negative large sized blasts with basophilic cytoplasm and cytoplasmic blebbing in almost all the cases (92%) that were CD41 positive with grade III fibrosis in 70% patients.

The FCM-IPT profile showed CD41a and CD61a positivity in 85% of cases along with CD-34, CD-117 and HLA-DR, whereas in 15% cases diagnosis was rendered on bone marrow biopsy immunostains. CD 34 expression was seen in all the cases.

42% and 64% cases showed dim to moderate CD13 and CD33 positivity respectively. Half of the cases (50%)cases showed HLA-DR positivity.14% showed CD7 aberrancy.CD41 positivity was seen in 85% cases and CD61 positivity in 92% cases. Two of the cases showed a RAM phenotype.

The cytogenetic studies showed abnormal karyotypes in 65% of cases which included MLL rearrangements (21%), complex karyotype (14%), and trisomy 8(1%) and BCR-ABL fusion (1%).

None of the cases except for one which was recently diagnosed had survived on a follow-up period of 6 months to 2 years.

Conclusion: AMKL patients have distinct morphology and immunophenotype fatures and are associated with high risk cytogenetics subtypes.

Anemia Including Hemolytic Anemia

MLASA-1: A Rare Cause of Sideroblastic Anemia with Myopathy

Benazer Sait, Aakash Chandran Chidambaram, Dinesh Babu R M, Krishnamoorthy Vidhyasagar, Joshua Rajan Xavier, Benjamin Sagayaraj

Introduction: Myopathy, lactic acidosis, and sideroblastic anemia-1 (MLASA-1) is a mitochondrial disorder that involves skeletal muscle and erythrocytic cell-line of the bone marrow. It is characterised by mutations in PUS1 gene, with varying severity of presentation. This mitochondrial deficiency disorder essentially involves the skeletal muscle and erythrocytic cell-line of the bone marrow. This disorder is an ultra-rare genetic disease with only 34 pathogenic and 11 likely pathogenic variants described in ClinVar as of July 2022.

Aims & Objectives: Herein, we describe a 3 year old girl who presented with severe failure to thrive, cardiomyopathy, transfusion dependent anemia, myopathy and lactic acidosis.

Materials & Methods: A 3-year-old girl, born to non-consanguineous parents, was brought with complaints of pallor, failure to thrive and floppiness of all limbs noted since early infancy. Her perinatal history was uneventful. There was a significant family history in the form of early sibling deaths. Her developmental milestones were delayed in all domains. She was admitted twice in the past for anemia, requiring blood transfusions. Her anthropometric parameters revealed severe undernutrition (weight-7.8 kg (< -3Z score); height-86 cm (< -3 Z score) and microcephaly (43 cm). General examination revealed severe pallor, mild facial dysmorphism in the form of frontal bossing, flat nasal bridge and high arched palate. Neurological examination revealed generalised wasting, hypotonia, muscle power of 4/5, exaggerated reflexes and flexor plantar response.

Result: The initial investigations revealed microcytic hypochromic anemia with increased serum lactate levels. Her serum iron and serum ferritin levels had improved on oral iron therapy but there was no concomitant increase in hemoglobin, arousing the suspicion of sideroblastic anemia. Echocardiography was suggestive of cardiomyopathy. Clinical exome sequencing revealed a novel homozygous missense variation in PUS1 gene suggestive of MLASA-1.

Conclusions: Knowledge about MLASA-1 adds to the existing literature on syndromes with sideroblastic anemia and myopathy.

Performance Charactersitics of High Performance Liquid Chromatography and Haemoglobin Capillary Zone Electrophoresis in Estimating HbA2 Levels

Gurpreet Kaur, Seema Tyagi, Tulika Seth, RenuSaxena, Manoranjan Mahapatra

Introduction: Haemoglobin A2 (HbA2) constitutes less than 3% of the total haemoglobin (Hb) and is an important determinant in the diagnosis of beta thalassemia trait (BTT).In some cases, the level of HbA2 is not typically elevated and some difficulties may arise in making the diagnosis therefore quantification of HbA2 has to be performed with accuracy. Cation exchange high-performance liquid chromatography (HPLC), and HbCZE (Haemoglobin capillary zone electrophoresis) are considered acceptable methods to diagnose BTT.

Aims & Objectives: In this study, we attempted to compare HbA2 values using two methods using HPLC and HBCZE.

Materials & Methods: 536 whole blood samples sent by physician-ordered haemoglobinopathy screening were studied. The performance characteristics of both these machines were compared for HbA2 the detection of the 5 most common haemoglobin variants i.e. (Hb A, HbF, HbS, HbC, HbE).

Result: On comparing the HbA2 values the HPLC showed higher values for HbA2 as compared to HbCZE while the Hb F, HbS measurement agreement was good between both methods. On comparing the HbA2 values the HPLC values for HbA2 had a median value of 2.9% as compared to HbCZE where the value was 2.65%. The correlation was better at lower HbA2 values than at higher values and the correlation improved once the HbE, HbC and HbS were excluded.

Conclusions: Normal ranges and means normal values of HbA2 differ between different methods hence each institute using these machines should validate their own cutoffs and as both these methods are complimentary, in cases of rare variants both HPLC and HbCZE should be carried out besides other ancillary techniques and molecular methods.

Hepatitis B Seroconversion Amongst Patients with Transfusion-Dependent Beta Thalassemia

Nirali Sanghvi, Priyanka Aggarwal, Vineeta Singh, Vineeta Gupta

Introduction: Children with thalassemia are particularly vulnerable to hepatitisB infection due to multiple transfusions and immunological derangements. The global prevalence rates for hepatitis surface antigen(HBsAg) positivity have been reported as 0.3–5.7% amongst children with Thalassemia.

Aims & Objectives: Aim of this study was to determine antiHBs antibody levels and its correlation with other factors in children with transfusion-dependent thalassemia(TDT).

Materials & Methods: A cross-sectional study was conducted amongst 60 children with TDT visiting our Thalassemia daycare and who had completed primary hepatitis B vaccination. The data was collected over a period of 1 year. Participants were assessed for anti-HBs titres at baseline and divided into 3 groups: groupA (titre < 10 miu/mL), groupB (10- < 100miu/mL), groupC (> = 100miu/mL). GroupA (seronegative) participants were administered full-course of Hepatitis B vaccine (at 0,1 and 6 months) and Group B participants

were given a single booster dose. They were evaluated for antiHBs titres following vaccination.

Result: Among 60 participants, 45 (75%) were males and 15 (25%) were females with mean age of 11.3 ± 5.09 years (range:4–26 - years). Homozygous beta-thalassemia was present in 42(70%) participants and 18(30%) had double heterozygous disease (including HbE-beta and HbS-beta thalassemias). In groups A, B and C there were 19 (31.7%), 16 (26.7%)and 25 (41.6%) participants each respectively. Amongst participants, 19(31.7%) were seronegative, while 41 (68.3%)were seroprotected(antiHBs titre > = 10miu/mL). Seroprotection rates for hepatitisB were significantly higher in younger participants (< = 5 years) than older(> 5 years) ones. All 8 younger children(100%) had protective titres as against 33(63.4%) out of 52 older children. Those participants with early initiation of transfusion before or at 2 years of age were more seroprotected than those with late initiation of transfusion after 2 years. Twenty-seven out of 34 participants(79.4%)with early initiation of transfusion were seroprotected as against 14 out of 26 partcipants(53.8%) with later initiation(p = 0.03). All the 19 seronegative children achieved seroprotection after immunization with hepatitis B vaccine.

Conclusions: Children with TDT should be regularly assessed for antiHBs antibody titres and immunized accordingly so as to maintain their seroprotected status against Hepatitis B infection.

TABLE: CLINICAL PROFILE OF PARTICIPANTS

CHARACTERISTICS		n=60 (%)	AntiHBs Titre<10 mIU /mL (n=19)	AntiHBs Titre>=10 mIU/mL (n=41)	p Value
GENDER	MALES	45 (75%)	14	31	0.8
	FEMALES	15 (25%)	5	10	
AGE	<=5 YEARS	8 (13.3%)	0	8	0.04
	>5 YEARS	52 (86.7%)	19	33	
DIAGNOSIS	HOMOZYGOUS BETA THALASSEMIA	42 (70%)	12	30	0.4
	DOUBLE HETEROZYGOUS THALASSEMIA	18 (30%)	7	11	
AGE AT INITIATION OF TRANSFUSION	<=2 YEARS	34 (56.7%)	7	27	0.03
	>2 YEARS	26 (43.3%)	12	14	

Role of Next Generation Sequencing in Chronic Hemolytic Anemia: A Single Center Experience

Shreya Gupta, Vineeta Gupta, Priyanka Aggarwal

Introduction: Hemolyticanemia is defined by premature destruction of erythrocytes. Hemolyticanemia may be classified as (Intrinsic/Extrinsic, Immune/Non immune, Acute/Chronic, Inherited/Acquired, Intravascular/Extravascular). They result from alteration in structure, transport and metabolic functions of red blood cells. Newer diagnostic tools like next generation sequencing (NGS) may help us to identify the genetic basis of these disorders.

Aims & Objectives: To determine the role of NGS in the diagnosis of cause of chronic hemolyticanemia.

Materials & Methods: All children during Jan 2020–August 2022 that presented to outpatient department with features of recurrent or chronic hemolyticanemia i.e. pallor, icterus and hepatosplenomegaly, and their routine investigations were inconclusive were subjected to NGS. The routine investigation were CBC, reticulocyte count, LDH, serum bilirubin (direct/indirect), coomb's test (direct/indirect), osmotic fragility, G6PD levels, urine routine microscopy for hemoglobinuria, general blood picture and high performance liquid chromatography.

Result: During the study period, 11 children presented with chronic hemolyticanemia that were subjected to NGS. Out of these, 8 were males and 3 females with mean age 7.5 ± 4.2 years. The most common symptom with which the child presented to us was progressive/recurrent pallor (n = 9) followed by yellowish discolouration of body (n = 8), and recurrent abdominal pain (n = 1). Osmotic fragility was increased in 54.5% children. On NGS, majority of children were diagnosed to have Hereditary spherocytosis type 2(AD) (n = 5), Non spherocytic glucose phosphate isomerase deficiency (AR) (n = 1), Pyruvate Kinase deficiency (n = 1) and, Hereditary Spherocytosis type 1(AR) (n = 1). We were not able to establish the cause of hemolyticanemia by NGS in three children although 2 among these 3 children had increased osmotic fragility.

Conclusions: NGS can be used as an adjuvant diagnostic tool for diagnosis of cause of chronic hemolyticanemia where definitive diagnosis can't be reached by routine conventional tests or where children are affected by rare hemolytic disorders.

Prominent Golgi Zone in Recovering Bone Marrow

Nitin Dayal, Rahul Naithani

Introduction: A 72 year male with history of rheumatoid arthritis with multiple joint deformities was admitted with syncope and gastrointestinal bleeding.

Aims & Objectives: He was taking methotrexate and hydroxychloroquine since last 7 years. Perioral excoriation and petechial spots over palate were present. There was no fever, lymph node enlargement or splenomegaly. Patient was given 3 doses of G-CSF and referred for hematology consult. Complete blood count showed pancytopenia. Absolute neutrophil count was 0.06 × 109/L and platelet count was 10 × 109/L. Red blood cells were normocytic normochromic with low reticulocyte count. Direct antiglobulin test was negative and LDH was normal.

Materials & Methods: Retrospective case report.

Result: Methotrexate was stopped. Bone marrow aspiration and biopsy showed hypercellular marrow with normoblastic erythroid maturation. Myeloid precursors were increased in number with many promyelocyte with prominent Golgi zone. Normal or reactive promyelocytes were characterized by prominent paranuclear clear Golgi zones. Megakaryocytes were adequate. No confluent blast aggregates or ALIP were seen. A diagnosis of methotrexate induced bone marrow suppression was made.

Conclusions: Patient's neutrophil counts and platelets showed dramatic recovery the very next day of bone marrow examination and remained normal thereafter.

A Rare Case of Complicated Malaria Presenting as Warm Autoimmune Hemolytic Anemia

Sonu Choudhary, Geetika Sharma, Shilpi More, Nimisha Sharma, Sujata Raychaudhuri, Tathagata Chatterjee

Introduction: India contributes to majority of the malaria burden of Southeast Asia globally (2%).Autoimmune HemolyticAnemia(AIHA) has been rarely reported worldwide as well as from India as the underlying cause of anemia in malaria. Recent insights regarding the pathogenesis of anemia in malaria has highlighted the role of humoral and cellular autoimmune response.

Aims & Objectives: A case report of complicated Plasmodium falciparum malaria with concomitant warm AIHA detected after meticulous transfusion reaction work up in the patient incidently.

Materials & Methods: ABO/Rh blood grouping, Direct Antiglobulin Test (DAT) and Indirect Agglutination Test (IAT) were performed by column agglutination technique (Gel Card method). Antibody

screening and identification was performed by 3 cell and 11 cell panel respectively.

Result: A 31-year old male, presented with episodes of high-grade fever, headache, easy fatigability and epistaxis. A blood requisition form for 3 units of Fresh Frozen Plasma (FFP) along with the EDTA and plain vial sample was received in the Department of Immunohematology and Blood transfusion. After transfusion of FFP, the patient developed itching, fever and rash. Detailed transfusion reaction workup was done. IAT antibody screen of the pre transfusion sample was negative. DAT of post transfusion sample was positive (1 +) with positive auto control (1 +). Further, on DAT screen with monospecific AHG, red cells were coated with IgG (1 +). The eluate from DAT positive red cells showed a pan-agglutination reaction. Peripheral smear examination showed crescent shaped gametocytes, single and multiple ring forms. Many accole forms of Plasmodium falciparum species were also seen along with occasional spherocytes (Parasitemia 12%).

Biochemical investigations showed raised LDH, SGOT, SGPT and hypoalbuminemia. The rapid malaria card antigen test was positive. Thus, diagnosis of warm AIHA in complicated malaria was suggested. Single unit of least incompatible (1 +). A Rh positive packed red cells was reserved for the patient. Patient was started on artesunate Injection Falcigo 2.4 mg/kg IV bolus. The patient was followed up closely for delayed post artesunate hemolysis till day 9.

Conclusions: This case highlights the fact that in patients of malaria with persistent anemia or sudden drop in hemoglobin levels, autoimmune hemolysis should be ruled out by careful re-evaluation of hematological, biochemical and immunohematological parameters. Autoimmune hemolysis can complicated the course of severe malaria and delay in recognition and appropriate treatment poses a significant burden on health expenditure.

Interaction of Alpha Gene Deletion and HO-1 Genotypes on the Phenotype of Indian Sickle Cell-Beta Thalassemia Patients

Hareram Pandey, Ravi Ranjan, Jasmita Dass, SeemaTyagi, Tulika Seth, Manoranjan Mahapatra

Introduction: Sickle cell β-thalassemia is a compound heterozygous condition of β- thalassemia and sickle cell anaemia. The phenotype with these conditions showed mild to severe clinical phenotype. In India, the frequency of the sickle gene reported 40% especially in the tribals, whereas the incidence of the β-thalassemia gene is around 3–4% in the general population. It has been reported that the coexistence of α-thalassemia with severe β-thalassemia results milder phenotype. The few studied have shown that coinheritance of haem oxygenase-1 (HO-1) in sickle cell disease patients modulate the HbF levels. We have found that very few studies addressed the effect of HO-1 polymorphisms (HO-1-413A/T (rs2071746) in SCD patients and hence, we planned this study.

Aims & Objectives: This study was aimed to evaluate the effect of α-deletion and understand the impact of HO-1 polymorphism on HbF level in Indian SCD patients.

Materials & Methods: 40 healthy controls and 40 confirmed cases of Sβ were recruited. Clinical, hematological, and molecular characterization was performed in all subjects. α- genotyping was performed by GAP-polymerase chain reaction while Allele Specific PCR was applied for HO-1 gene polymorphism. Data were expressed as mean ± SD. The differences among the various hematological parameters within the genotypes of polymorphism were calculated by ANOVA, using Graph Pad Prism version 3.06. P value < 0.05 was taken statistically significant.

Result: Out of 40 Sβ, 14 (36%) were with α-chain deletion. Individual who had α-deletions were improved clinical features.The highest frequency of α-3.7 heterozygous (8 patients) followed by α-3.7 homozygous (4 patients), while two patients had α-triplication. Out of 40 Sβ, 16 (40%) were heterozygous, 13 (32%) were homozygous and 11 (28%) were wild type. Clinical severity was improved with variant genotype in SCD patients. The HbF level was found higher in variant (TT) genotype than wild type.

Conclusions: The TT genotype of the rs2071746:A > T polymorphism was associated with increased levels of Hb F ($P < 0.001$). It can serve as a HbF modifier in Indian Sickle Cell diseases patients.

Hodgkin's Lymphoma Presenting as Auto Immune Haemolytic Anemia

Sambangi Ravichand, Deepak Gautam, Atique, Bhargava Chaitanya, Subhash Yadav

Introduction: Autoimmune haemolytic anaemia (AIHA) can be either primary (idiopathic) or secondary associated with an underlying disease such as lympho-proliferative disorder, autoimmune disease or infection. Lymphoproliferative disorders especially include chronic lymphocytic leukemia and non-Hodgkin's lymphoma but it is rarely seen in Hodgkin's lymphoma(HL).

Aims & Objectives: To discuss a case of Hodgkin's lymphoma presenting as AIHA and the intricacies in it.

Materials & Methods: A 28 year old male patient presented with the history of fever, generalised weakness, weight loss, loss of appetite for 1 month. On general examination there was severe anaemia, icterus, multiple palpable cervical and axillary lymph nodes which were non tender and of rubbery consistency. Systemic examination revealed splenomegaly.A complete blood count showed macrocytic anemia with hemoglobin of 4.6 g/dl, mean corpuscular volume of 116 fl and reticulocyte count of 23%. Direct Coombs test was strongly positive for IgG and C3. Bilirubin was raised at 5.4 mg/dl with elevated indirect bilirubin of 4.3 mg/dl and lactate dehydrogenase of 964U/l. A diagnosis of warmAIHA was kept. Subsequently, the patient underwent lymph node biopsy, which revealed HL mixed cellular type and came positive for cd15 and cd30. A whole-body ct-scan showed cervical, axillary,mediastinal and abdominal lymphadenopathy. A final diagnosis of warmAIHA associated with Hodgkin's lymphoma stage 3B was made. Patient was started on oral prednisone 1 mg/kg body weight, Rituximab(100 mg/week × 4) and ABVD regimen.

Result: One month later repeat complete blood counts showed improving haemolytic anaemia. Chemotherapy for Hodgkin's lymphoma is still going-on.

Conclusions: When HL is accompanied by AIHA, the hemolysis is usually detected at the time of diagnosis or a relapse. Immune mediated hemolyticanemia is mostly seen in nodular sclerosing subtype and in mixed cellularity subtypes.In majority of these patients, AIHA is associated with clinical or pathological evidence of stage III or stage IV disease, but it does not necessarily worsen the outcome of Hodgkin's lymphoma.Although the initial treatment of AIHA is steroids, immune haemolysis associated with Hodgkin's disease requires definitive treatment with systemic chemotherapy. Patients with AIHA refractory to steroid treatment should be worked up for any underlying malignancies.

Anaemia as the Presenting Feature of an Underlying Occult Malignancy (Metastatic Adenocarcinoma of Prostate)

B Sai Bhargava Chaitanya, Lalit Prashant Meena, Arun Kumar Singh, Subash, Ravi Chand, Atique

Introduction: A variety of tumours can lead to bone marrow metastasis.

Due to the frequency of these tumours in men, the cause is often prostate cancer or lung cancer (especially small-cell lung cancer) and breast cancer in women.

Although anaemia associated with advanced prostate cancer has been reported, But presentation only with anaemia is very unusual.

Aims & Objectives: To report an unusual case,which had anaemia as the only presenting feature of Metastatic adenocarcinoma of prostate.

Materials & Methods: A 60 year old male patient presented with Generalised weakness and easy fatiguability for 1 year

General examination showed moderate to severe pallor rest including systemic examination including per rectal examination was within normal limits

General blood picture showed normocytic,normochromic anaemia of HB-8.5 g/dl,MCV-90 fl,MCHC-33 g/dl with anisopoikolocytosis with teardrop cells, microcytes, macrocytes

Fe/TIBC/Ferritin—87 mg/dl/338/230 mg/dl; serum LDH-230 U/L; corrected reticulocyte count-2.2%

LFT showed ALP- 970u/l and rest of the parameters including GGT are normal

Further Bone marrow Aspiration had been tried from sternum and iliac crest but it was unsuccessful probably due to the underling hard bone(Difficulty in penetration of aspiration needle)

Bone Marrow biopsy showed markedly thickened,woven bony trabeculae in mosaic Pattern with Teleroid appearance and very little marrow elements

Inter trabecular spaces are compressed and shows tri lineage depression of haemopoiesis

Haemopoietic tissue is replaced by vascular connective tissue

Reticulin stain—(MF-0)

Differentials were: Paget's disease of bone, Osteosclerosis, Tumor Osteopathy

In the view of the above suspected diseases Tc99-MDP Bone scan was performed

Scan features are suggestive of Extensive Osteoblastic Skeletal metastases

Pattern suggestive of Metastatic super scan

Then serum Total PSA performed shows— $>$ 1000 ng/ml (normal range $<$ = 4)

Free PSA— $>$ 30 ng/ml (normal range is 0–0.5)

TRUS guided prostate biopsy reveals: Adenocarcinoma Gleason score (3 + 4) Peri neural invasion is present

Patient was treated for metastatic prostatic carcinoma with Bicalutamide, Leuprolide and Abiraterone

Result: The anaemia of the patient responded to the treatment with a current haemoglobin of 12gm/dl.

Conclusions: Anemia is not a diagnosis;
it is a manifestation of an underlying disorder.

Thus, even mild, asymptomatic anemia should be investigated so that the primary problem can be diagnosed and treated.

Autoimmune Hemolytic Anemia in A Case of Rheumatoid Arthritis

Dilshad Khan, Amitava Mazumdar, Prantar Chakrabarti

Introduction: Autoimmune hemolyticanemia (AIHA) is a decompensated acquired hemolysis caused by the host's immune system acting against its own red cell antigens. AIHA caused by infection, malignancy, autoimmune, drugs, etc.Different types of anemia can affect people with RA like anemia of chronic disease, iron deficiency anemia, drug induced etc. The incidence of autoimmune hemolyticanemia in RA is uncertain, but it has been estimated to be 2.1–2.5%.

Aims & Objectives: To find out the etiology of anemia in a patient with RA.

Materials & Methods: 78 Year old female admitted with RA with joint flare was found to be anemic and investigatedfurther.Patient did not have any history of blood loss or fever.Onexamination,there was no lymphadenopathy and hepatosplenomegaly. Patient had swelling, tenderness of all small joint(bilateral) with deformity.She was on Methotrexate, folate and NSAIDS.

Result: Investigation showed other hematological parameter Hemoglobin-7.8 g/dl total leucocyte count 4300 cells/cmm, Platelet count = 140,000/microL, iron = 72 mcg/dl, ferritin = 513mcg/L, corrected reticulocyte count 4.1%,LDH = 537U/L and DCT = 4 + .Vitamin B12 and Folate normal range, stool OBT negative.CRP = 21.4 mg/dl.ANA = negative.RA = High titre positive.She responded to corticosteroid therapy.

Conclusions: Anemia in RA can be multi factorial and needs to be worked up for identifying the causes and AIHA is a rare association with RA patient.

Clinical Profile and Outcome of Autoimmune Hemolytic Anemia In Infants

Swathi Krishna, Sangeeta Mudaliar

Introduction: Autoimmune hemolyticanemia (AIHA) is an immunologic disorder in which antibodies are produced that target red blood cells (RBCs). The annual incidence in children is reported to be 1–3 cases/100,000 patients. There is scarcity of data on infants with AIHA, their incidence and their treatment outcomes. We present data of 4 Infants with AIHA and the challenges involved in their treatment.

Aims & Objectives: To analyse clinical and laboratory parameters and treatment outcomes of infants with autoimmune hemolyticanemia (AIHA).

Materials & Methods: Retrospective analysis of 4 infants in whom direct antiglobulin test (DAT) and investigations for secondary AIHA were performed and clinical, laboratory and treatment outcomes were analysed.

Result: The median age at diagnosis was 12 months with all infants being male. All the infants presented with severe anemia, congestive cardiac failure and hepatosplenomegaly. In 3 infants the direct antiglobulin test was positive, 3 of them had reticulocytopenia at the time of presentation, 1 infant was diagnosed with Evans' syndrome, 2 infants had post-infectious AIHA secondary to CMV Virus and Parvo virus. Immunological cause was identified in one patient through next generation sequencing(NGS). Initial Treatment modalities included steroid, intravenous immunoglobulin(IVIg) and steroid with IVIg. After a median follow-up of 2 years, 3 infants had achieved remission at 17 months with second line drugs like Azathioprine.

Conclusions: Infants presenting with AIHA may require ICU care in view of severe anemia and hypoxia. It is not commonly diagnosed due to lack of clinical suspicion as well as unavailability of appropriate diagnostic tools in infants to rule out secondary causes. Monospecific DAT and a thorough search for an underlying cause can help optimize therapy. Managing AIHA in infants and those with secondary AIHA is challenging, with almost all of them at our center needing second line agents like Azathioprine to achieve remission.

Aplastic Anemia Following SARS-COV-2 Infection

Swathi R, Akanksha Bhatia, Vijay Kumar

Introduction: COVID 19 is caused by a novel virus SARS-CoV-2.It has become a pandemic as declared by WHO with its first case being reported in China. Among children the intensity is usually mild and without any further impact.

Aims & Objectives: Unusual presentation of aplastic anemia following SARS-CoV-2 infection: A rare case report.
Materials & Methods: A 6 year old male child presented with complaints of rashes and epistaxis for 2 weeks and also one episode of blood in stools.Two weeks prior to the onset of above complaints, the patient had a history of recovery from COVID -19.Blood investigations revealed pancytopenia with hemoglobin of 5.6 gm/dl,total leukocyte count of 2000/cumm and platelets were 43,000/cumm.The corrected reticulocyte count was 0.3%.Bone marrow examination done showed completely hemodilutedsmears.Bone marrow biopsy revealed a markedly hypocellular marrow with cellularity of 10% and the cellular components being replaced by fat spaces.
Result: Based on the above findings, and other viral markers being negative a diagnosis of aplastic anaemia following SARS CoV-2 was made.
Conclusions: COVID-19 being a relatively new disease,it's sequelae in children is not much studied.Aplasticanemia following an infection of SARS-CoV-2 is extremely rare with only two cases reported in literature till date.Hence this entity should be kept in mind by the treating physician encountering a case of pancytopenia following COVID-19.

Pharmacological Correction of Human Genetic Glucose-6-Phosphate Dehydrogenase Enzymopathy for Clinical Management of Oxidative Stress Induced Hemolytic Anemia

Nutan Gupta, Shreeja Biswas, Nishant Joshi, Swati Garg, Soumya Pati, Shailja Singh

Introduction: G6PD deficiency is a genetic metabolic abnormality which is caused by deficiency of the enzyme G6PD. The gene encoding G6PD are found on the distal long arm of the X chromosome. It is a key and rate limiting enzyme in the pentose phosphate pathway (PPP). It contributes to many chronic diseases associated with the oxidative stress. Infection, certain foods, and medication cause oxidative stress. Moreover, treatment of malaria eradication programme involves the generation of ROS, thus this knowledge/cure decreases the fatality risk rate. Approximately 500 million people are affected worldwide. Currently no medications are available to cure G6PD deficiency, thus current study seek to identify drug like small molecule that corrects it.
Aims & Objectives: (1) Expression, purification and functional study of G6PD enzyme variants.
(2) Screening of small molecule as an agonist of G6PD activity.
Materials & Methods: All variants were expressed in ecoli c43 strain and purified using affinity chromatography. All small molecules were tested at 0.01 mM conc.
Result: Melatonin was earlier reported to increase the activity of wild type Human G6PD enzyme. So Melatonin and its derivatives were tested, if they increase the activity of the G6PD mutant variant too. We started with canton and Mediterranean variants. As reported Melatonin was found to increase the activity of G6PD wild type enzyme by 22.6% while it increases the activity of canton and Mediterranean variants by 39.22% and 59.52% respectively. Melatonin derivative K 185 (k1888) was found to increase activity of wild type enzyme by 26.7% while of canton and Mediterranean variants by 36.7% and 55.58% respectively. 6-Hydroxymelatonin (H0627) increases wild type enzyme activity by 27.25% and of canton and Mediterranean variants by 42.85% and 52.99%. N-Acetyl-5-hydroxytryptamine (A1824) increases wild type enzyme activity by 22.47% and of canton and Mediterranean variant by 26.3% & 40.63%. Melatonin and derivatives were tested for their antimalarial activity too to explore whether they can be used as potent molecules for Malaria eradication since it take cares of G6PD deficient condition and antimalarial action both.
Conclusions: Melatonin & derivatives found to increase the activity of G6PDd alleles.

Thalassemia Intermedia Co-Existing with Coeliac Disease

Alka Yadav, Neha, N D Vaswani

Introduction: Both celiac disease and Thalassemia intermedia can present as microcytic hypochromic anemia refractory to iron therapy but the extent of association is less known.
Aims & Objectives: Aims and objectives: We are reporting this unusual case with co-inheritance of thalassemia intermedia and celiac disease and to emphasize the fact that delay in the diagnosis of one or the other condition, will impact the management and overall health of these children.
Materials & Methods: Patient D, 10 years old male was referred to us for short stature and poor weight gain over the last 4 years. He was diagnosed with a case of thalassemia intermedia at four and a half years of age based on the clinical and laboratory findings. HPLC done at that time was showing HbF 6.8% and HbA2 10.1%. Both the parents were thalassemia traits with HPLC as mother (HbA 66.5%, HbF 0.2%, HbA2 25.9%), father (HbA 82.2%, Hb F 0.6%, HbA2 6.0%). The child had received multiple transfusions (12–13 in total) from the local hospital since diagnosis.
Result: At the time of presentation child was found to be short-statured (height 115.6 cms i.e. $<$ 3rd centile). There was severe pallor and hepato-splenomegaly, spleen measuring 10.3 cm below costal margin on examination. There was dimorphic anemia on PBF with Hb 6.6%, TLC 11,400, P39, L55, M3, B3 with a corrected reticulocyte count of 6.1%. Serum ferritin at enrollment was 2034.7 ng/dl. The patient was put on put on regular transfusion regimen along with chelation therapy in view of significant growth failure. Other investigations to look for causes of short stature were performed and found normal. The patient was diagnosed as a co-existing celiac disease based on the serum tTG A level of 50.3 u/ml and duodenal biopsy suggestive of partial villous atrophy. The patient was started on a gluten-free diet along with hydroxyurea at a dose of 23 mg/kg/d and a three-weekly transfusion regimen. Three months into the follow up the child is clinically well with a height velocity of 1.51 cm per month.
Conclusions: Conclusions: Coeliac disease can coexist with thalassemia resulting in growth faltering and severe anemia. Putting these patients on a gluten-free diet along with hydroxyurea might help in improving the outcome.

Mean Reticulocyte Volume (MRV) Adds Value in the Diagnosis Of Hereditary Spherocytosis as a Rapid Screening Test

Nitty Skariah Mathews, Venkatesh Dhanasekaran, Rutvi Gautam Dave, Tulasi Geevar, Joy John Mammen, Sukesh Chandran Nair

Introduction: Hereditary spherocytosis (HS) is an inherited hemolytic disorder due to a red cell membrane defect. Complete blood count and reticulocyte counts are often the first tests ordered in the evaluation of a suspected haemolytic disorder. Making a diagnosis of HS is a clinical problem as there are no specific diagnostic tests. Conventional tests for HS such as osmotic fragility test (OFT) have low sensitivity and is tedious to perform. Some red cell and reticulocyte parameters have been found useful in the evaluation of HS.

Aims & Objectives: The aim of this study was to demonstrate the utility of mean reticulocyte volume (MRV), a simple parameter available on several automated haematology analyzers in the detection of HS.
Materials & Methods: In this prospective study, 112 suspected HS cases and 120 healthy voluntary blood donors (controls) were enrolled. Complete haemogram, reticulocyte count, peripheral blood smear examination, direct antiglobulin test, osmotic fragility test (OFT), and cryohaemolysis were performed on all cases and controls. Haematological parameters of all study participants were measured on Beckman Coulter DxH 800 automated haematology analyzer.
Result: Among 112 cases, 60 were diagnosed as HS and 18 were autoimmune haemolytic anaemia (AIHA). MRV was less than the mean corpuscular volume (MCV) in 53/60 (88.3%) HS cases, in contrast to 2/18 (11.1%) AIHA cases and none among the controls ($p < 0.0001$). MRV was significantly lower in HS cases than in controls ($p < 0.0001$) and AIHA ($p < 0.0001$). At an MRV cut-off of 91.05 fL for a diagnosis of HS, the area under the ROC curve was 0.998 with 95% CI [0.993, 1.000] corresponding to a sensitivity, specificity, positive predictive value and negative predictive value of 100%, 98.3%, 99.2%, and 100% respectively.
Conclusions: MRV is a simple, quick, inexpensive and readily available parameter to screen for HS. We provide an MRV cut-off value to detect HS with excellent sensitivity, specificity, and high predictive value.

A Rare Hemoglobinopathy: Hemoglobin Monroe

Sushma Yendamuri, Uday Yanamandra

Introduction: When a patient presents with a long term history of anemia and waxing waning jaundice,we consider hemoglobinopathies, red cell membrane defects and enzyme defects as differential diagnosis.Additional history of recurrent blood transfusions make the diagnosis towards hemoglobinopathies.However a patient diagnosed with hemoglobinopathy becomes transfusion free we must revisit the original diagnosis and undertake further evaluations.This case is one such interesting case.
Aims & Objectives: to discuss the common presentation of rare haemoglobinopathy.
Materials & Methods: A 23-year-old male presented with complaints of easy fatigability, waxing and waning jaundice, yellowish discolouration of sclera and dragging sensation in left hypochondrium since childhood. He also received monthly blood transfusions (52) from age of 8-15 years, being transfusion free for last 5 years. He was diagnosed as a case of beta thalassemia major based on HPLC 2008 (Hb F 96.4%,and HbA2:2.7
Result: Beta thalassemia is one of the most common hereditary diseases in Mediterranean region comprising of 280 mutations. These mutations will lead to absence or reduction of beta globin chains resulting in beta-Thalassemia phenotype.The Haemoglobin Monroe results from a splice site point mutation in the last nucleotide of the β-globin exon 1. Till date, a hand full of cases of Hb Monroe have been diagnosed and reported, first from USA and later from Bangladesh, Tunisians, Tajiks, South of Iran and India. In our case he was initially diagnosed a beta thalassemia major by HPLC. While the diagnosis of beta thalassemia major explains the patients' symptoms, it should also render him transfusion dependant for life. However, the patient was transfusion free for 5 years with plateau hemoglobin of 10 g/dl. Therefore genetic analysis was done, homozygous mutation for Hb Monroe.
Conclusions: Mutation analysis in this case resulted in a change in the diagnosis and impacted the treatment protocol. With out genetic study,the patient would've continued receiving blood transfusions that he did not need with the concurrent risk of developing secondary hemochromatosis.

Consumer Compliant Herbal Beverages of Kulekhara for Anemia Correction

Roshnara Mishra, Anusua Singh, Raghwendra Mishra

Introduction: The most conventional treatment of anemia correction is iron supplementation which has its own shortcomings such as GI disturbance, etc. Hence, development of alternative treatment modalities is warranted such as herbal remedies.
Aims & Objectives: The aim of this study is to prepare modified herbal beverages of fresh *Hygrophila spinosa* leaf extracts not only as an effective anti-anemic drug but also to increase its popularity in terms of its palatability by aiming consumer compliance.
Materials & Methods: Fresh leaves of kulekhara were processed to prepare Kulekhara herbal tea (KHT) and kulekhara herbal tea fermented with kombucha (kombu-kk). The total phenolic content, flavonoid content, antioxidant capacity and constituent analysis of the variants were measured and compared with fresh kulekhara extract. An experimental animal model for inflammatory anemia (AI) has been developed by inducing turpentine oil and confirmed by a battery of hematological parameters. The effect of herbal beverages in correcting anemia in the animal model was studied. Human volunteers were enlisted in this study for the sensory evaluation to check the overall acceptability of kulekhara recipes.
Result: The KHT and kombu-kk showed comparable flavonoid, polyphenol and anti-oxidant content with fresh kulekhara extract. The HPLC analysis of both the herbal recipes revealed the presence of a variety of phytochemicals, some of which have known hematinic activity. All the phytochemicals were found to be present in the preparations when compared with the fresh kulekhara extract. The oral supplementation of Kombu-kk and KHT results in significant alteration of hematological markers in the animal model. This result indicates that the hematinic potential of kulekhara was well preserved in the modified recipes. Evaluation of the sensory value of kulekhara preparations, when compared with fresh kulekhara extract, showed the highest overall acceptability. Kombu-kk was greatly accepted followed by KHT. The herbal extract of fresh kulekhara showed the least acceptability in sensory value.
Conclusions: From this study, it can be concluded that the phytoconstituents and bio-activity of kulekhara were preserved in both KHT and Kombu-KK preparations and they effectively cure inflammatory anemia in animal model. Sensory evaluation revealed the overall acceptability of the preparations. A detailed study in a larger population is warranted.

Kulekhara Herbal Tea Treatment Attenuates Anemia of Inflammation in Murine Model and Corrects Dysfunctional Erythropoiesis

Anusua Singh, Tuphan Kanti Dolai, Roshnara Mishra, Raghwendra Mishra

Introduction: Anemia of Inflammation (AI) is characterized by bone marrow suppression associated with enhanced extramedullary hematopoiesis (EMH). Although there are various drugs for the treatment of AI, but they are not readily available in major parts of the world. In the last few years, herbal medicine is gaining popularity for prevention/cure of a variety of ailments. Kulekhara (KK) is one such ethnomedicinal plant which is used as a home remedy for the treatment and prevention of anemia. The scientific validation of hematinic activity of KK and its mechanism of action is still lacking.

Aims & Objectives: The present study aims to validate the anti-inflammatory and hematinic potential of aqueous extract of fresh Kulekhara (FKK) leaves and processed kulekhara herbal tea (KHT) for the treatment of AI in an experimental murine model.
Materials & Methods: The herbal tea of FKK and KHT were prepared using decoction method. An experimental AI model was developed by subcutaneous injection of turpentine oil and anemia was confirmed by a decline of total hemoglobin by $\geq$ 2 gm/dl. After the confirmation of AI, the treatment groups were given the herbal tea preparations per orally and animals were sacrificed after treatment period and hematological parameters, inflammatory markers and iron profile were estimated. Cell cycle analysis and Ter-119/CD-71 based immunophenotypic classification of bone marrow (BM) and splenic hematopoietic progenitors were conducted to evaluate the hematinic potential of herbal tea.
Result: AI is confirmed by a significant alteration of inflammatory markers, total hemoglobin concentration and plasma iron content associated with marked discoloration of the red bone marrow in the turpentine oil group. Both FKK and processed KHT treatment, significantly improved/normalized all the hematological indices and iron profile. In treatment group, the red color of bone marrow was also replenished suggesting correction of bone marrow dysfunction. CD-71/Ter-119 expression-based immunophenotyping yields a significant reduction in late erythrocyte progenitors in the bone marrow and higher splenic erythropoiesis indicating dysfunctional erythropoiesis & EMH- a hallmark of AI group. This condition was significantly reversed both in BM and spleen indicating normalisation of erythropoiesis in FKK and KHT treated groups.
Conclusions: From the above results, it can be concluded that both fresh and processed kulekhara herbal tea preparation possesses comparable hematinic as well as anti-inflammatory potential. The processed kulekhara leaves can be used for long-term preservation and for herbal tea preparation and can be consumed for hematinic benefits.

Next-Generation Sequencing Reveals the Rare Hyper-Unstable Hemoglobin Showa-Yakushiji Masquadering as Classical ?-Thalassemia Trait on Hb Hplc

Ravina Taak, Manu Jamwal, Namrata Singh, Prashant Sharma, Jasbir Kaur Hira, Deepak Bansal, Arindam Maitra, Reena Das

Introduction: Hemoglobinopathies are Mendelian disorders caused by defects in the biosynthesis of α or β-globin chains. They present a diverse phenotype that ranges from asymptomatic to transfusion-dependent symptomatic anemia. Hb Showa-Yakushiji is one of the β-globin variant that presents as mild hemolyticanemia (HA).
Aims & Objectives: We describe a child with recurrent unconjugated hyperbilirubinemia and HA who was found to have a rare unstable Hb variant, Hb Showa-Yakushiji on next-generation sequencing.
Materials & Methods: A 7-year-old female from Gaya, Bihar, presented with recurrent unconjugated hyperbilirubinemia, anemia, and mild hepatosplenomegaly. She had no history of blood transfusions. The complete blood count (CBC) showed hemoglobin (Hb) 76 g/L, and the reticulocyte count was 5%, as shown in Table 1. The peripheral blood smear showed mild anisopoikilocytosis, microcytic hypochromic red cells, with few elliptocytes, and teardrop cells. The CBC and blood smear were suggestive of classical β-thalassemia trait (βTT). The incubated osmotic fragility, G6PD screening, and direct antiglobulin (Coombs) test were normal. The Hb high-performance liquid chromatography (Hb-HPLC) was consistent with a classical βTT, HbA2 (5.6%) with mildly elevated HbF (5.4%). Molecular testing of [TA] repeats in the promoter region of UGT1A1 and multiplex Gap PCR for α-globin deletion and triplication was done. Whole exome sequencing (WES) was performed using the Nextera Rapid Capture Enrichment kit. Validation of identified variant was done using Sanger sequencing.
Result: Molecular testing of [TA] repeats revealed homozygous [TA]7/7, suggesting Gilbert's syndrome. The patient was negative for α-globin deletion and triplication for α-thalassemia. WES revealed a heterozygous hyper-unstable variant Hb Showa-Yakushiji NM_000518.4(HBB):c.332 T > C (p.Leu111Pro) in exon 3 of the HBB as the pathogenic variant.
Conclusions: The Hb Showa-Yakushiji was first reported by Kobayashi et al. in 1987 in Japanese family being investigated for βTT. This variant is a rare hyper-unstable Hb variant. The Leu to Pro substitution at residue codon 110 of the β-globin chain disrupts the G helix and the α1β1 contact of the Hb molecule. This leads to an unstable Hb variant with a thalassaemic phenotype. This case highlights the importance of genetic testing of globin genes in cases where Hb-HPLC reveals a classical β-TT pattern but clinical symptoms are discordantly severe. Unstable Hb can be falsely increase HbA2% and therefore be misdiagnosed as typical βTT, as exemplified in our case.

Hematological Manifestations in Patients with Systemic Lupus Erythematosus

Satya Prasad Namala, P Prabu

Introduction: Systemic Lupus erythematosus (SLE) is a chronic autoimmune disorder which affects multiple organ systems. Various haematological manifestations seen in SLE. There are lacunae of knowledge regarding exact prevalence of these abnormalities and their association with disease activity, organ involvement. Understanding prevalence and patterns hematological manifestations of SLE can help to provide inputs for prompt diagnosis and correction.
Aims & Objectives: To find out the prevalence of hematological abnormalities and the relation between severity of hematological abnormalities and disease activity (assessed by SLEDAI score) in patients with SLE.
Materials & Methods: This is a prospective observational study of patients aged $\geq$ 18 years with SLE; those with known primary hematological disease are excluded. Relevant demographic, clinical and laboratory parameters were documented. Patients were divided into two groups: SLEDAI score $\leq$ 10 and > 10. Relation between severity of hematological abnormalities and disease activity was assessed.
Result: Women aged between 20–29 years were most affected. Hematological abnormalities were detected in 90.4% of patients. Anemia was the most common hematological abnormality. Iron deficiency (40%) was the most common cause followed by anemia of chronic disease (29.6%) and autoimmune hemolyticanemia (12.2%). Severe anemia was associated with renal involvement ($p = 0.023$). Leucocytopenia was detected in 26.1% of patients. Lymphocytopenia was most common white cell abnormality with mean lymphocyte count of 1226.85 cells /mm3. Thrombocytopenia was detected in 23.5% of patients, associated with neurological involvement ($p = 0.023$). Anti dsDNA antibodies were associated with renal involvement ($p = 0.028$). Anticardiolipin antibodies were associated with anemia ($p = 0.006$) and renal involvement ($p = 0.003$). Low C3 ($p = 0.002$), simultaneous low C3, C4 ($p = 0.004$) were associated with serositis. Mean haemoglobin concentration ($p = 0.011$), lymphocyte count ($p = 0.046$) and C3 levels ($p = 0.0001$) were low in patients with severe disease activity. There was a significant correlation between severe anemia and disease activity ($p = 0.006$). Serositis, vasculitis, nervous system and renal involvement was high in patients with SLEDAI > 10.
Conclusions: There is a strong need for large-scale prospective studies to understand the prevalence of hematological abnormalities and its impacts on disease severity and various outcomes.

Hematological abnormalities may occur in majority of SLE patients, and are associated with certain disease manifestations and organ involvement.

Hemophagocytic Lymphohistiocytosis (HLH) In Visceral Leishmaniasis: A Diagnostic Dilemmma

Samir R. Agarwal, Vrinda Kulkarni

Introduction: Hemophagocytic Lymphohistiocytosis (HLH) is a syndrome of pathologic immune activation characterized by clinical signs and symptoms of extreme inflammation.
Aims & Objectives: Often the principle challenge for treating patients with HLH is making a timely diagnosis. It is also critical to search for and treat underlying triggers of HLH, and institute specific antimicrobial therapy.
Materials & Methods: A patient presented with Pancytopenia, on bone marrow was diagnosed as HLH and treated with HLH 2004 protocol but did not respond. On repeat marrow was diagnosed with Visceral leishmaniasis, whic was the trigger for HLH.
It is also critical to search for and treat underlying triggers of HLH, and institute specific antimicrobial therapy.
Result: Diagnosing HLH is the first critical step toward successful therapy but is challenging because of.
the rare occurrence, variable presentation, and nonspecific findings of this disorder.

Primary HLH

- clear familial inheritance or genetic causes.
- infants or younger children,
- To have fixed defects of cytotoxic function.
- clear risk of HLH recurrence.
- not likely to survive long-term without HSCT.

Secondary HLH

- older children (or adults).
- without a family history or known genetic cause.
- typically have concurrent infections/medical conditions that appear to trigger their HLH, such as
- EBV infection,
- malignancy, or.
- rheumatologic disorders.
- risk of recurrence in cases of secondary HLH is poorly defined.

Conclusions: Hemophagocytic lymphohistiocytosis can be diagnosed if there is a mutation in a known causative gene or if at least 5 of 8 diagnostic criteria based on HLH-2004 protocol are met.
1. Fever (peak temperature of $> 38.5°$ C for > 7 days) 2.Splenomegaly (spleen palpable > 3 cm below costal margin) 3. Cytopenia involving > 2 cell lines (hemoglobin $< 9 > 177$ mg/dL [2.0 mmol/L] or > 3 standard deviations [SD] more than normal value for age) or hypofibrinogenemia (fibrinogen $< 150 > 3$ SD less than normal value for age) 5. Hemophagocytosis (in biopsy samples of bone marrow, spleen, or lymph nodes) 6. Low or absent natural killer cell activity 7. Serum ferritin > 500 ng/mL (> 1123.5 pmol/Lng/mL) 8. Elevated soluble interleukin-2 (CD25) levels (> 2400 U/mL or very high for age).

Molecular and Clinical Influence Of HMOX1 Polymorphism on the Indian Sickle Cell Disease Patients

Hareram Pandey, Ravi Ranjan, Sumanlata, Jasmita Dass, Seema Tyagi, Tulika Seth, Manoranjan Mahapatra

Introduction: Sickle cell disease (SCD) is seen often in India, a chronic life-long disorder that begins in childhood and characterized by substitution of adenine for thymine at codon 6 of the β-globin gene. HMOX1 gene, present on the long arm of chromosome 22, codes the hemeoxygenase 1 enzyme and this gene is over expressed in sickle cell patients. Few studies have shown that polymorphisms of the HMOX1 gene, including rs2071746: $A > T$, cause an increase in the HbF level possibly lowering the disease severity, but in India, very less studies are conducted with this polymorphism, hence, we planned this studies.
Aims & Objectives: This study was aimed to evaluate the role of HMOX-1 polymorphism on HbF level in Indian Sickle cell disease patients.
Materials & Methods: Fifty sickle cell disease patients were recruited. Clinical details were noted. CBC was measured. HPLC was performed to characterize SCD (HbSS&Sβ). Screening of HMOX1-413A/T (rs2071746) SNP was performed using allele-specific PCR. Data was expressed as mean ± SD, the differences among the HbF levels within the genotypes of polymorphism were calculated by ANOVA. P value < 0.05 was taken as statistically significant.
Result: A total 50 healthy controls and 50 SCD (25SS & 25Sβ) cases were characterized. Most of the cases of HbSS had normocytic, normochromic anaemia and those of HbSβ were microcytic, hypochromic anaemia. HMOX1 polymorphism was studied in all subjects. Out of 25 HbSS patients, 11 (44%) were heterozygous (AT), 8 (31%) were homozygous (TT) and 6 (25%) were found wild type (AA) genotype. Out of 25 HbSβ heterozygous patients, 10 (40%) were heterozygous, 8 (32%) were homozygous and 7 (28%) were wild type. Clinical severity was improved with variant genotype. HbF level was found higher in variant (TT) genotype and was statistically significant (p-value < 0.001).
Conclusions: This study indicates that co-existence of HMOX-1 rs2071746:$A > T$ polymorphism increased the levels of HbF and ameliorate the Indian phenotype.

Congenital Dyserythropoietic Anemia Type II with SEC23B Mutation: a Rare Entity

Archana Samal, Priyanka Samal, Samir Sahu

Introduction: Congenital dyserythropoieticanemia type II (CDAII) is an autosomal recessive form of hereditary anemia caused by SEC23B gene mutations. Patients with CDA present in early life with anemia, jaundice, splenomegaly, gall stone, iron overload and a suboptimal reticulocyte response for the degree of anemia due to ineffective erythropoiesis. Type-II is the most common form of CDA, the red cells are lysed by acidified serum therefore the disease is also known as hereditary erythroblastic multinuclearity with positive acidified serum lysis test (HEMPAS).
Aims & objective: To diagnose a case of longstanding history of anemia of unknown etiology in a 17 year old boy.
Case report: A 17 year old boy, presented to us with a history of anemia since childhood. At age of 4 year, he has evaluated for low hemoglobin level & was diagnosed as Hemolytic anemia, but workup for the common types of hemolytic anemia was negative. Patient was managed conservatively with blood transfusion, folic acid, hepatoprotective drugs for raised bilirubin.
Patient visited us at 17 years age with Hb 2.7 gm/dl, reticulocytosis-10.07% and other parameters in near normal range. Repeat tests for Hemolytic anemia done was negative, bone marrow done outside was suggestive of marked erythroid hyperplasia with minimal dysplasia, 46XY karyotyping and in view of strong clinical suspicion, Clinical exome sequencing was done from peripheral blood which reported SEC23B mutation- common mutation in CDA type II. The patient had received a total of 6 units till 17 years age and had mild icterus. On

examination- there was no facial dysmorphism, anthropometric parameters were normal, severe pallor and mild icterus. His liver was 3 cm palpable and spleen was 4 cm enlarged. Serum Ferritin was 3420 ng/dl inspite of the infrequent blood transfusions, suggesting increased dyserythropoiesis, as in thalassemia intermedia. Repeat Bone marrow Biopsy was done, but there was no typical morphological dysplasia as described in CDA type II.

Conclusion: CDA-II is a rare congenital anemia. Patients may remain undiagnosed due to variability in severity of anemia and lack of typical morphological features in Bone marrow. NGS helps to establish a diagnosis in such cases. Early identification of CDA II is important to prevent secondary iron overload and end organ damage.

Blood Biochemical Parameters in Beta Thalassaemia Patients Of Odisha

Bhagyalaxmi Das, Rabindra Kumar Jena and Bisnu Prasad Dash

Introduction: The occurrence of different Haemoglobinopathies in Odisha has been known since 1952. Several reports on distribution and hematological aspects of sickle cell disorders and beta thalassemia have been documented. Majority of the Beta Thalassemia patients are surviving with frequent blood transfusion hence subjected to excess iron overload. This may leads to organ dysfunction and premature death.

Aims & Objectives: Keeping in view of this, a study was conducted to know the plasma biochemical parameters of sixty two Beta Thalassemia major patients (Male: 40, Female: 22) attending a tertiary medical center of the state.

Materials & Methods: The identification of the cases was done following the Bio Rad High Performance Liquid Chromatography using beta thalassemia short programme. All the serum biochemical parameters were estimated by the standard procedures adopted in our laboratory.

Result: The mean age of the patients was 9.3 + 4.4 years. Only four patients were above 20 years of age. The mean serum total and direct Bilirubin was found to be 1.82 + 0.98 mg/dl and 0.55 + 0.39 mg/dl respectively. The mean Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were found to be 464.33 + 194.22 U/L, 67.82 + 36.92 U/L and 66.94 + 65.14 U/L respectively. The mean serum urea and creatinine values were 24.08 + 4.80 mg/dl and 0.72 + 0.16 mg/dl respectively. The age of onset of first symptom of the studied cases varied from 3 months to 4 years. The mean serum ferritin level of the studied cases was 3162.20 + 2556.41 ng/liter.

Conclusions: Majority of the beta thalassemia patients were under 20 years of age. All the serum biochemical parameters in the patients showed abnormalities even if patients were under regular blood transfusion regimen. The abnormal serum biochemical parameters indicate the dysfunctions of different vital organs of the body. However further studies are needed with larger patients of different age, sex and areas to understand the basic pathophysiology of the Beta Thalassemia patients of Odisha state.

A Simpler Cost-Effective Rapid Qualitative Method for the Diagnosis Of G6PD Deficiency Using Whole Blood

Rati Mukesh Devendra, Puloma Pandey, Tejashree Anil More, Manisha Madkaikar, Rucha Patil

Introduction: Rapid test for Glucose-6-phosphate dehydrogenase (G6PD) are essential for determining G6PD deficiency, a widespread metabolic disorder which triggers haemolytic anaemia in response to oxidative drugs. Current gold standard diagnostic tests (quantitative assay using UV spectrophotometer and DPIP tube test) for G6PD detection are although cost-effective, both need instruments, need -20 °C for storage of reagents and technical expertise.

Aims & Objectives: To develop a simple, sensitive, cost-effective, rapid qualitative technique for the diagnosis of G6PD deficiency using whole blood with no instrument requirement.

Materials & Methods: The test standardized has three solutions: solution A, B and C. 1 drop (10ul) of whole blood is added to solution A, mixed, incubated for 5 min at room temperature and then 1 drop of solution B followed by 5ul of solution C has to be added. Color change occurs instantly; color remains the same as that of the haemolysate (bright red) when G6PD is sufficient. If it is brownish black and bluish black then its G6PD intermediate or deficient respectively. So, 10 samples each: G6PD deficient, normal and intermediate; confirmed with the gold standard method were used to standardize this simple assay.

Result: The internal validation of the test was done on known G6PD normal (N = 60), G6PD deficient (N = 20) and G6PD Intermediate (N = 11) blood samples. The sensitivity and specificity were found to be 100%. External validation is yet to be done. The reagents in all the solutions have been stabilized at 4 °C for ten months. Further, our method does not require specialized instruments making it more efficient over the gold standard technique. This method could easily be deployed for screening large number of samples as a rapid screening test. The reduced number of steps and the rapid time for the determination of enzyme deficiency further suggest its high-throughput accuracy.

Conclusions: The newly developed qualitative test is proposed to be an alternative to the existing standard techniques. Our method could be used for the population screening program. Quick diagnosis among the vulnerable groups will further help the clinicians at the PHCs to provide better prophylaxis (Fig. 1).

Autoimmune Haemolytic Anaemia: A Rare Complication of Malaria

Lohitha Bhavani Jasthi, Priyanka Samal, Samir Sahu, Aswini Kumar Sahoo

Introduction: Close to 1.3 billion people are at high risk of being infected with malaria in India. India carries 2% of the global malaria case burden and 2% of global malaria deaths. Autoimmune Hemolytic Anemia, very infrequent condition which represents a group of disorders characterized by presence of autoantibodies directed against self-antigens leading to shortened red cell survival. Till date, a very few cases of AIHA in Malaria patients are reported worldwide but still AIHA should be considered a relatively rare cause of anemia in malaria.

Case report: A 26 year female was admitted with c/o fever and yellowish discoloration of sclera for 10 days.Pallor and Icterus were present. Patient was treated with artesunate injection in view of malaria and she received 4unit PRBC for anemia. In view of no increment in hemoglobin and high Retic count, hemolytic work up was done. She had DCT (4 +) auto-control (4 +),10% reticulocyte count, deranged LFT and raised LDH (1111) favoring a diagnosis of AIHA. Other causes of autoimmune condition were ruled-out. We further confirmed the case as AlHA due to her response to corticosteroid following which Hb improved from 7.3 to 12gm% after 2 weeks without further transfusion.

Discussion: Anemia is a frequent association with malaria, usually by destruction of RBCs by parasites, splenic sequestration, dyserythropoiesis, increase in inflammatory cytokines and nutritional deficiency. Here, patient was suffering from high grade malarial parasitemia at the time of admission with co-existing autoimmune RBCs destruction by IgG auto-antibody which led to sudden drop in Hb, increase in

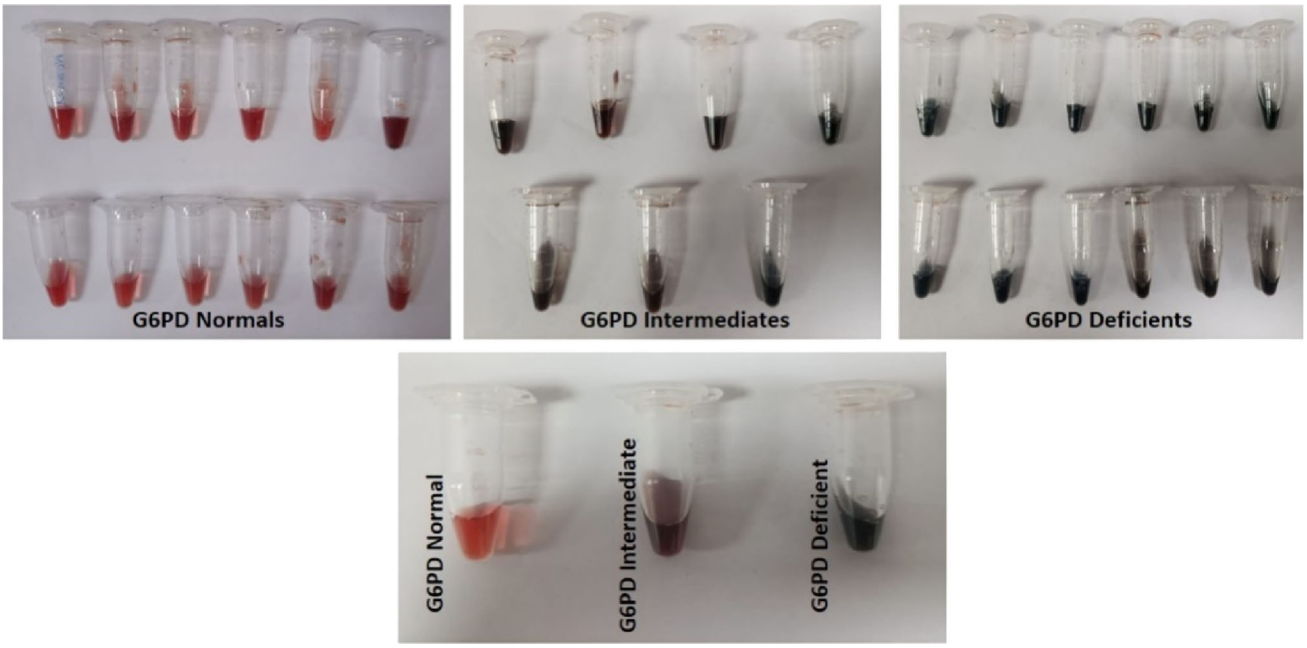


Fig. 1 Microtube method for the detection of G6PD deficiency

serum indirect bilirubin and serum LDH. The incidence of AIHA is rare in India, affecting one to three people per 100,000 per year.
Conclusion: If malaria patient presents with anemia and jaundice, AIHA should be considered and further evaluation must be done.

Homozygosity for Delta-Beta Thalassemia & Hereditary Persistence Of Fetal Hemoglobin—An Uncommon Cause Of Elevated Hemoglobin F: A Tale Of Two Cases with Review

Vaka Ramesh, Praptiaacharya, Chinamayee Panigrahi, Somanath Padhi, Gaurav Chhabra

Introduction: Delta-beta (δβ) thalassemia or Hereditary persistence of fetalhemoglobin(HPFH) is an uncommon cause of raised fetalhemoglobin (HbF) after infancy. It is characterized by reduction in production of both δ and β-globin chains, usually due to deletions of δ and β structural genes. The heterozygotes for δβ-Thalassemia and HPFH are clinically asymptomatic or present with mild anemia. However, very rarely it may present as homozygous for δβ-thalassemia and HPFH with moderate anemia and organomegaly giving a clinical picture of thalassemia intermedia and may be misdiagnosed as beta thalassemia major.
Aims & Objectives: We describe two rare cases of Homozygosity for Delta-Beta thalassemia or Hereditary persistence of fetalhemoglobin and their clinical and hematological features with a literature review.
Materials & Methods: Two cases, aged 24 years and 15 years old male presented with complaints of generalized weakness and yellowish discoloration of the eyes. The clinical examination revealed pallor, icterus, splenomegaly in both the cases. The family history was negative in both the cases, with one patient being an orphan, and siblings being completely asymptomatic. Further on the laboratory workup, the CBC revealed mild to moderate anemia with reduced red cell indices. The peripheral smear showed microcytic hypochromic red cell morphology with anisopoikilocytosis showing target cells, tear drop cells and pencil cells with polychromasia. There was reticulocytosis, hyperbilirubinemia, normal iron studies. Based on this a diagnosis of hemolyticanemia was made and hemolytic workup was done, the Coombs test was negative and Hb HPLC revealed markedly elevated Hb F of more than 90% in both the cases. The parental screening was done that too revealed Hb F of 27 and 23% in father and mother respectively. Based on the finings the final diagnosis of homozygous for Delta-Beta thalassemia or homozygous HPFH was made in both the cases and DNA analysis was advised for confirmation.
Result: δβ-thalassemia are relatively uncommon form of thalassemia, and are characterized by lack of β and δ-globin chain production. This reduction in production is usually caused by deletion of δ and β structural genes. Homozygotes for δβ-thalassemia or HPFH usually present with greater than 90% HbF resulting from of the increased synthesis of HbF, and usually present with a beta thalassemia intermedia like clinical phenotype or may be completely asymptomatic too. Since on HPLC it presents with markedly raised Hb F, it become very important to differentiate it from beta thalassemia major for the further management of these cases. Parental screening and molecular studies along with clinical presentation forms the mainstay of the correct diagnosis.
Conclusions: This case highlights the importance of considering homozygous δβ-thalassemia or HPFH in presence of elevated HbF and normal or slightly reduced HbA2. The clinical and haematological findings along with family and transfusion history helps in differentiating these rare disorders from other causes of raised Hb F such as Beta thalassemia major or even in the compound heterozygous states of beta thalassemia with other Hb variants.

Importance of Evaluation of Alpha Thalassemia Mutation Highlighted by A Case of Iron Refractory Microcytic Hypochromic Anaemia

Deepsikha Bhanja, Khushboo, Manali, Siyaram Didel, Abhishek Purohit

Introduction: Iron deficiency anaemia (IDA), β-thalassemia trait (βTT) and anaemia of chronic disease (ACD) are common causes of microcytosis that can be diagnosed accurately by Iron studies and haemoglobin high performance liquid chromatography (Hb-HPLC) respectively. However, when HbA2 level is normal or low along with normal or low serum iron studies, microcytosis can be a diagnostic dilemma. Cases of microcytosis not responding adequately to iron supplementation are diagnostic dilemma and have been reported to harbour alpha thalassemia mutations.
Aims & Objectives: Aim of presenting this cases report is to highlight keeping high index of suspicion while dealing with microcytic hypochromic anaemia. To study and do comprehensive workup of a case of iron refractory microcytic hypochromic anaemia for alpha thalassemia evaluation considering future prospects for the society.
Materials & Methods: The present case was seen in Haematology clinic in collaboration with Department of Paediatrics and Department of Pathology & Lab Medicine at All India Institute of Medical Sciences, Jodhpur. Case was evaluated with complete history, sequential haemogram findings, peripheral smear examination, morphology, supravital staining, Hb-HPLC and molecular genetic findings.
Result: A 17-year old girl with complaint of chronic fatigue, dizziness, poor weight gain and microcytic hypochromic anaemia was evaluated in Haematology clinic. Patient had received adequate iron supplementation. Other causes of microcytosis was also evaluated. Hb-HPLC revealed low HbA2 (1.6%), suspected HbH (1.8%), normal HbF and HbA. Supravital staining for HbH inclusions revealed presence of golf-ball inclusions in red cells. On genetic testing, for alpha thalassaemia gene analysis for large comprehensive copy number variations in HBA1 and HBA2 genes, pathogenic variant was identified for alpha thalassemia.
Conclusions: Alpha gene mutation can confound iron deficiency anaemia, but no red cell indices, or discriminant function can identify. For all cases of microcytic hypochromic anaemia with normal iron studies and iron refractoriness, comprehensive workup for alpha thalassemia should be done and proper genetic counselling should be advocated for future implications.

Outcome of Prenatal Diagnosis by Chorionic Villus Sampling in Pregnant Women with Thalassaemia Trait: A Retrospective Study from a Tertiary Care Centre in West Bengal

Chirasree Sanyal, Kaustav Ghosh, Shipla Roy, Abhishek Maurya, Apurva Baneerjee, Subham Bhattacharya, Shuvra N. Baul, Sandeep Saha, Prakash K Mondal, Rajib De, Tuphan K. Dolai

Introduction: Thalassemia and other hemoglobinopathies display high level of molecular and clinical heterogeneity and are quite common in eastern India. It has been noted 0.37 out of every 1000 foetus may have haemoglobinopathies. It can be prevented by population and antenatal screening, genetic counselling, offering prenatal diagnosis and by selective Medical Termination pregnancy (MTP).
Aims & Objectives: To asses outcome of prenatal diagnosis by Chorionic Villus Sampling in pregnant women with thalassaemia trait.
Materials & Methods: This retrospective study was done from July 2021 to August 2022. Prenatal diagnosis was done by chorionic villus sampling (CVS). Antenatal screening was done with HPLC (Biorad Variant II Short Thalassemia programme) for antenatal mother and spouses of thalassaemic trait mother. In case of both parents are found to be thalassaemic trait, CVS study followed by mutation analysis done by ARMS PCR in the Department of Haematology at NRSMCH.
Result: A total of 150 pregnant females underwent CVS. The mean maternal and paternal age mean 26 yr (± 4.15) & 31 yr(± 5.32) respectively. The gestational mean age was 14 weeks (± 2 week) at the time of CVS sampling. HPLC analysis shows 67% and 33% mother were Beta thalassemia trait and E trait, respectively and70% and 30% of the father were Beta thalassemia trait and E trait respectively. Following CVS 33.2% foetus were diagnosed affected (homozygous and compound heterozygous) undergone MTP after appropriate counselling. Out of 66.8% of all CVS diagnosed as normal or trait, counsel to continue pregnancy. The genotype study revealed IVS1-5 (G > C) followed by CD26(G > A) are most common mutations in parents who underwent CVS testing and no complication was recorded during procedure. Most common genotype was IVS1-5 (G > C) 56.3% and 49.7% in affected and carrier foetus.
Conclusions: By genetic counselling, PND and selective MTP 33.2% thalassaemic child birth was prevented in one year in our department. So Prenatal Diagnosis is safe, essential and effective step to prevent thalassaemic birth.

Characterization of Microcytic Hypochromic Anemia in Children from Southern Odisha, India

Shailasuta Das, Prasanta Purohit, Samira Kumar Behara

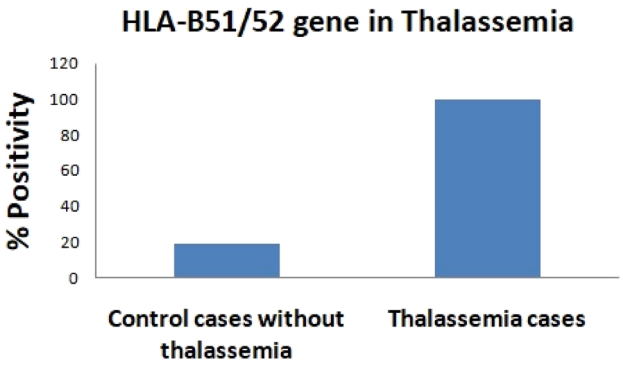
Introduction: Anemia is common in children affecting around 50% of children in India. Majority of anemia cases are microcytic-hypochromic anemia and may characterized with iron deficiency (ID), inheritance of thalassemias (both α thalassemia and β thalassemia), certain vitamin deficiency etc. Though the preliminary diagnosis of β thalassemia can be done by chromatography techniques, the final diagnosis can only be performed by molecular methods. Similarly, α thalassemia can only be investigated by molecular methods.
Aims & Objectives: To investigate the prevalence of α thalassemia and β thalassemia in children diagnosed with microcytic-hypochromic anemia.
Materials & Methods: The study was conducted in the Multi-Disciplinary Research Unit (MRU) and Department of Pathology of M.K.C.G. Medical College, Berhampur, Odisha. Children with microcytic-hypochromic anemia attending to this hospital were investigated for hemoglobin variants using Variant-II (Bio-Rad), serum ferritin by auto-analyzer, and α-thalassemia (α-3.7 & α-4.2 deletion) by Multiplex PCR.
Result: A total of 115 children diagnosed with microcytic-hypochromic anemia were included. The median age was 5 years with 50.4% being females. The analysis of hemoglobin variants revealed 43 (37.4%) children with abnormal hemoglobin disorders including 19 cases with β-thalassemia trait, 10 cases with sickle cell trait, 6 cases with sickle cell anemia, 3 cases with sickle cell-β-thalassemia, 4 cases with β-thalassemia major and 1 case with hemoglobin E trait. From the serum ferritin analysis, 34 (29.6%) cases had low serum ferritin level/ID. Out of 115 cases, diagnosis of α-thalassemia was successful in 114 cases with a prevalence of 53.5%. Both heterozygous and homozygous α-thalassemia was detected with a prevalence

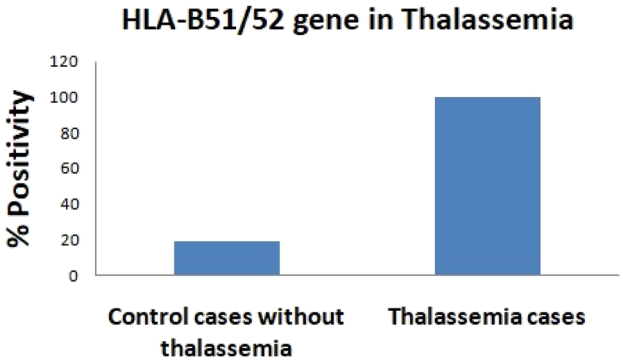
of 35.1% and 18.4% respectively. On analysis, the microcytic-hypochromic pictures remain uncharacterized in 9 cases.
Conclusions: This study confirms that α-thalassemia is the most common cause of microcytic-hypochromic anemia compared to iron deficiency and β-thalassemia. Further, the significant prevalence of both hemoglobin disorders (α-thalassemia and β-thalassemia) in the population calls for a molecular diagnostic approach to microcytic-hypochromic anemia.

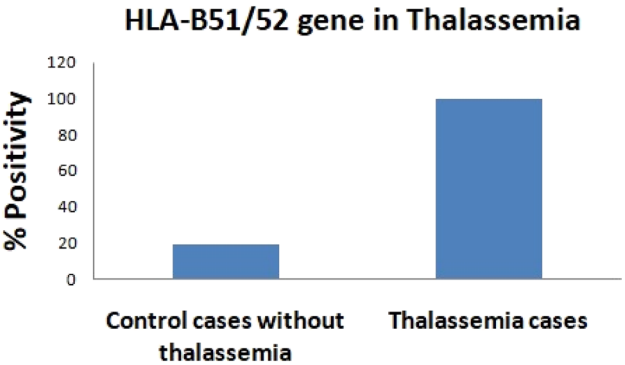
Mutation Profile of Hemoglobinopathies and its Association with Clinical Phenotype at a Tertiary Care Centre in Eastern Uttar Pradesh

Priyanka Aggarwal, Akhtar Ali, Nirali Sanghavi, Vineeta Gupta

Introduction: Introduction: β thalassemia and Sickle cell disease are chronic hemolytic anemias that are inherited in an autosomal recessive manner and are caused by mutation in the beta globin gene cluster on chromosome 11.
Aims & Objectives: Aims & Objectives: To determine the mutation profile of children with hemoglobinopathies and its association with clinical phenotype.
Materials & Methods: Materials and Methods: This was a retrospective cross sectional observational study. All children with transfusion (TDT) and non-transfusion dependent thalassemia (NTDT) along with sickle cell disease were enrolled. The children were divided into 2 groups: Transfusion dependent (Group I) and Non transfusion dependent (Group II). The Group I, was further divided based on age at first blood transfusion i.e. $\leq$ 2 years (Group IA), $>$ 2 years (Group IB).
Result: Results: Total 156 patients with mean age 14.5 $\pm$ 8.8 years and M:F ratio 2.4: 1 were enrolled. Group I (n = 129), comprised of homozygous β-thalassemia (97) followed by Eβ-thalassemia (n = 21), Sβ-thalassemia (5), sickle cell anemia (2), β-δβ thalassemia (2), HbD-βthalassemia (1) and β-HPFH (1). In group IA (n = 83) among 8 homozygous and 16 compound heterozygous mutation types, the most common mutation was homozygous c.92 + 5 G > C (IVS 1–5) (n = 35) followed by c.92 + 5G > C/c.51del (IVS1-5/CD31) (n = 5), and c.92 + 5G > C/CD30 (IVS 1–5/CD30) (n = 5). In group IB (n = 46), among 6 homozygous and 15 compound heterozygous mutation types, the most common mutation was c.79G > A/c.92 + 5G > C (HbE/IVS 1–5) (n = 12) followed by homozygous c.92 + 5 G > C (n = 7). Group II (n = 27), included sickle cell anemia (n = 19) followed by HbEβ-thalassemia (n = 3), Hb SD (n = 2), HbD-βthalassemia (n = 2) and HbD Iran-β thalassemia (1). In this group, homozygous c.20A > C (HbSS) was discovered in majority of children (n = 19). The presence of homozygous c.92 + 5 G > C, c.79G > A/c.92 + 5G > C statistically predicted (p = 0.000) clinical phenotype as thalassemia major and thalassemia intermedia respectively.
Conclusions: Conclusion: Various mutations have been identified that are imperative for genetic counselling. However, c.92 + 5 G > C (IVS1/5) remains the most common mutation responsible for transfusion-dependent and c.20 A > C for non-transfusion-dependent clinical phenotype in our population.







A Possible Association of HLA-B 51/52 Gene with Heterozygous Beta Thalassemia

Debasmita Chatterjee, Banhishikha Singh, Krishnendu Paira, Satadal Das

Introduction: Human Leukocyte Antigen is a set of proteins which plays certain important functions in relation to our immune system. The antigen is coded by the genes present within chromosome number 6. In Indian population we have approximately 6.8 to 15% positive of HLA-B*51/52 among varied ethnic population. Beta-thalassemia is considered to be the most common autosomal disorder in the world having more than 535 mutations within the HBB gene.
Aims & Objectives: In the present study, we investigated a possible association of HLA-B*51/52 gene with β thalassemia.
Materials & Methods: In this randomized study, 65 control subjects, 10 patients with heterozygous β thalassemia were included in the study. The participants were informed about the purpose of the research study, thereafter, those who agreed to participate in the research investigation, 2 mL of blood samples were collected by a

trained phlebotomist within EDTA vials, after signing the informed consent. Whole blood genomic DNA extraction was carried out followed by Real Time PCR for the detection of HLA-B*51/52 gene.
Result: The findings revealed that out of 65 controls, 12 cases were found to be RT PCR positive for HLA-B*51/52 gene, 10 out of 10 heterozygous β thalassemia cases were found positive.
Conclusions: A strong association of HLA-B*51/52 with thalassemia is evident from the results of this preliminary study. However, a detailed study with larger sample size is necessary for the final conclusion.

Three Missense Mutations in SEC23B Gene Causing Congenital Dyserythropoietic Anemia Type Ii in the Indian Population

Arati Nandan Saptarshi, Rashmi Dongerdiye, Tejashree Anil More, Prabhakar Kedar

Introduction: Congenital dyserythropoietic anemias (CDA) are a rare group of inherited disorders characterized by ineffective erythropoiesis and dyserythropoiesis. CDA type II is caused by mutations in the *SEC23B* gene that encodes secretory COPII component. The diagnosis of CDA is difficult because of its rarity and unavailability of detailed protocol for diagnosis. Many cases of CDA type II get misdiagnosed as cases of hereditary spherocytosis because CDA type II cases show low mean fluorescence intensity (MFI) in the eosin-5'-maleimide (EMA) test.
Aims & Objectives: To diagnose and differentiate CDA type II & Hereditary Spherocytosis cases using biochemical test and molecular characterization of CDA type II using Sanger sequencing.
Materials & Methods: We have performed Eosin -5'-Maleimde (EMA) test followed by anti-CD44 antibody binding test for the diagnosis and differentiation of cases of CDA type II and Hereditary spherocytosis. Clinical details and family history of all patients is taken and molecular characterization and prediction of severity of variants using bioinformatics tools is performed.
Result: 13 patients were found to have low MFI in EMA and raised anti-CD44 antibody binding. They were confirmed to be cases of CDA type II based on molecular characterization using Sanger sequencing. All patients showed persistent anemia, icterus, pallor, and hepatosplenomegaly. Peripheral blood smear showed anisopoikilocytosis, few spherocytes, and hypochromic microcytic erythrocytes. The bone marrow analysis of 5 patients showed erythroid hyperplasia with mild dyserythropoiesis, bi-/multinucleated erythroblasts, and karyorrhexis in light microscopy. Molecular characterization was performed by Sanger sequencing and prediction of severity of variants by bioinformatics tools is given in table 1. According to genetic analysis, c.1385 A > G (p. Tyr462Cys) is the most common mutation found in the Indian population.
Conclusions: For the diagnosis of CDA type II, an EMA test followed by an anti-CD44 antibody binding test is helpful. Molecular diagnosis using Sanger sequencing and Next generation sequencing will help in prenatal diagnosis.

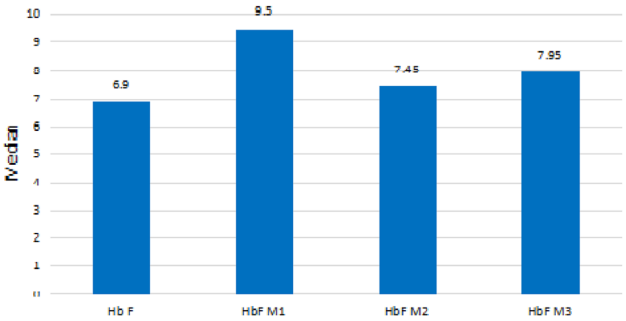
Table 1: Molecular characterization and prediction of severity of variants by bioinformatic tools

Sr. No.	Mutation Reported	Amino Acid Change	Zygosity	PolyPhen	SIFT	PROVEAN
1 N=11	c.1385 A>G	p.Tyr462Cys	Homozygous	Possibly damaging (0.689)	Damaging (0.00)	Deleterious (-6.048)
2 N=1	c.938 G>A	p.Arg313His	Homozygous	Probably Damaging (1.000)	Damaging (0.00)	Deleterious (-4.654)
3 N=1	c. 490 G>T	p.Val164Cys	Homozygous	Possibly Damaging (0.870)	Damaging (0.010)	Deleterious (-0.270)

Safety and Efficacy of Lenalidomide in Patients with Transfusion Dependent E-Beta Thalassemia Refractory to Hydroxyurea

Prerna Pramanik, Maitreyee Bhattacharyyaa

Introduction: Hemoglobin (Hb)-F inducers are known to improve Hb level and transfusion dependence in thalassemia. Thalidomide, a fetal hemoglobin (HbF) inducer that promotes γ-globin gene expression, has been reported to be effective for ß-thalassemia. There have been various studies showing effectiveness of thalidomide in TDT patients. Lenalidomide, an analogue of thalidomide was shown to have synergistic effect with hydroxyurea in the induction of fetal hemoglobin. There have been few reports of lenalidomide use in thalassemia.
Aims & Objectives: To evaluate the safety and efficacy of lenalidomide in patients with transfusion dependent E-Beta thalassemia refractory to hydroxyurea.
Materials & Methods: It is a prospective institution based study. Patients received 5 mg daily lenalidomide for 3 months. Primary end points were increment in Hb, Hb-F level and improvement in transfusion requirement; secondary end point were tolerability and safety. Response criteria was defined according to hemoglobin increment and Hb increment > 1.5gm/dl was taken as complete response.
Result: 19 patients were included, of which 11 were male and 8 female. The mean Hb at baseline was 6.9 ± 0.97gm/dl, the median HbF was 6.9 (2.1–21.7). At the end of 3 months, There was no increment in Hb, however there was increment in HbF level, the median HbF being 7.95(25–36.8). There was decrease in transfusion requirements in the majority of patients. Common adverse effects were itching and neutropenia(mild). Two of them discontinued therapy for intractable itching, others were managed with supportive management.
Conclusions: Lenalidomide resulted decrease in transfusion requirement in majority of transfusion dependent patients however there was no hemoglobin increment unlike thalidomide which has shown significant improvement in previous studies. The adverse effect profile of lenalidomide was satisfactory.



Clinico-Hematological Spectrum and Serological Characterization of Autoantibody in Patients of Warm Autoimmune Hemolytic Anemia: Experience from a Tertiary Care Government Hospital in Haryana Region

Geetika Sharma, Sonu Choudhary, Shilpi More, Nimisha Sharma, Saroj Rajput, Anjali Chauhan, Tathagata Chatterjee

Introduction: Autoimmune Hemolytic Anemia (AIHA) is a rare heterogenous group of diseases caused due to disturbance in immune homeostasis with autoantibodies directed against self-antigens on the red cells. In warm AIHA (w-AIHA), the antibodies react optimally at 37 °C and majority have red cell autoantibody IgG plus complement.Agglutination at room temperature is present in about one-third of patients with w-AIHA.
Aims & Objectives: To study the clinical, hematological and biochemical parameters in patients of warm AIHA and to serologically

characterise the red cell bound autoantibodies with regard to antibody class, DAT strength and titers.

Materials & Methods: The prospective analysis was conducted in the Department of Immunohaematology and Blood Transfusion at E.S.I.C Medical College and hospital,Haryana over a period of one year. ABO- Rh blood grouping & Direct Antiglobulin test (DAT) was done using gel card method. Antibody screening & identification was done by indirect antiglobulin test by using 3 -cell panel and 11- cell panel.Elution studies & pappain treatment were carried out on case basis. Antibody titers were done for the cold agglutinin in the saline phase at 4 °C.

Result: Eleven clinically suspected cases of AIHA showed male preponderance (72.72%) and the age varied from 7 months to 49 years. Majority were categorized as secondary AIHA (8/11, 72.72%).Splenomegaly was more common than hepatomegaly in both primary and secondary AIHA. In secondary AIHA, infectious etiology was seen in 4 cases (Mycobacterium tuberculosis, CMV, E.coli & Malaria) and 2 cases were of multiple myeloma and thalassemia major with concomitant hepatitis B & C respectively. One case each were relapsed hodgkin lymphoma, chronic liver disease, autoimmune vasculitis and disseminated malignancy.

Majority of the cases were diagnosed as warm AIHA (72.72%) while 3 cases had presence of insignificant cold agglutinins.(27.27%). DAT was positive in 9/11 cases while negative in 2 cases. The most common autoantibody immunoglobulin class was IgG (72.72%) and 3 cases had concominant C3d (27.27%).

Conclusions: It is of paramount importance to accurately subclassify the serological type for better patient management and arranging the best compatible units in these patients. Though majority of w- AIHA cases are primary, it is important to diagnose and treat the underlying cause in addition to steroid and immunosuppressive therapy.

Hematology in the Road To Motherhood, Hematological Parameters in Patients Visiting ART (Assisted Reproductive Technology) Centre

Solanki Darshansinh Nirusinh, Uday Yanamandra, Harshit Khurana, Abha Khurana, Gurpreet Kaur, Anil S Menon

Introduction: Infertility is a clinically common disease, occurring in approximately 10% of women of childbearing age. There is a paucity of studies investigating hematological parameters among infertile women and the association of anemia with infertility.

Aims & Objectives: To estimate the prevalence of anemia and the haematological profile among women undergoing infertility treatment.

Materials & Methods: This is a cross-sectional single centre descriptive study from Western India. Patients visiting artificial reproductive (ART) centre aged between 18–50 years and were cohabitating with husband for at least six months during the study period (Jan 2021 to Sep 2022) were screened for inclusion. Patients with chronic kidney disease, chronic liver disease, malignancy, on chemotherapy for any malignancy, taking drugs interfering with Iron/Vitamin B12/Folic acid metabolism and diabetes mellitus were excluded from the study. All included patients were subjected to CBC using 7-part coulter and those with any haematological abnormality were further assessed by haematologist for establishing firm diagnosis. Those patients who didn't give consent for detailed evaluation or those who were lost to follow up because of COVID were excluded from the study. Data was analysed using JMP ver 16.0.0.

Result: Study population included 108 patients with a mean age of 28.30 ± 4.14y. Eighty percent of the study population were home makers. Average annual family income was 7.55 ± 1.47 lakhs per year. The mean duration of infertility being 5.95 ± 3.52 years with most of them having primary infertility (81%). RBC characteristics of study population being, Hb- 12.12 ± 1.07 g/dL, MCV-81.57 ± 7.12fL, PCV—36.72 ± 2.88%, RBCs—4.52 ± 0.45million/μL, MCH—26.99 ± 2.86 pg, MCHC-33.02 ± 1.11 g/L, and RDW-14.96 ± 2.17. Other hematological profiles of the infertility patients revealed a total leukocyte count of 7439 ± 1897/μL, Platelets-2.53 ± 0.90/μL. Of all the patients 43% had anemia at presentation to the infertility clinic. Among those who had anemia 52% had microcytic hypochromic whereas 46% had normocytic normochromic with 2% having macrocytic anemia. Among those with microcytic hypochromic anaemia, the mean Mentzer index was 15.53 ± 2.40. Irrespective of anemia status, 30% of patients had microcytosis. Most of the patients with microcytic hypochromic anemia had Iron deficiency with one two patients having BTT. The prevalent anemia was not higher than the background prevalence. None of the patients had any leukocyte or platelet disorders.

Conclusions: There is no predominant haematological association with female infertility.

Microcytic Anaemia Factor (MAF): Is It A Magic Formula? Comparison Between Maf and Conventional Iron Markers in Geriatrics Age Group: A Case–Control Study

Deepak Dubey, Aditi pandey

Introduction: Iron deficiency anaemia (IDA)is one of the prevalent causes of anaemia globally. With variable and sometimes without any symptoms and often coexist with other causes of anaemia like Anaemia of chronic disease(ACD). Identification of iron status is paramount in the treatment of IDA.

Aims & Objectives: To evaluate the utility of Microcytic anaemic factor (Maf) as a single factor for reflection of iron status and its correlation with serum ferritin (S.ferritin), Interleukin -6 (IL-6), C-Reactive Protein (CRP) and hepcidin (HA).

Materials & Methods: Case–control Hospital-based study, conducted on individuals aged 60 to 80 years At District Hospital Bilaspur, 100 participants are included in this Study 50 Case groups with anaemia and 50 control group without anaemia. A one-time blood sample is collected and was evaluated for CBC, MAf, Serum Creatinine, S. ferritin, B12, Hepcidin, IL-6, and CRP.

Result: Primary Anaemia in the geriatrics age group was Anaemia of chronic disease with Maf Value ranging from 8.20 ± 1.71, Iron deficiency anaemia MAf value 6.99 ± 0.90. Where in megaloblastic anaemia Maf ranges from10.21 ± 1.58 With Conventional markers also show statically significance with Maf which include Hepcidin, CRP, IL-6, and ferritin with p-value < 0.01.

Conclusions: This study shows that MAf Can be used as the single calculation for the identification of iron status in the geriatric age group with Maf value < 6.00 being a definitive indicator for iron supplementation.

Burden and Epidemiological Factors Contributing to Iron Deficiency Anaemia in Children Between 6 Months To 5 Years Age: An Experience in a Tertiary Care Hospital

Surajit Bhattacharjee, Tarapada Ghosh, Prasenjit Sadhukhan, Sangey Nyngpo Bhutia

Introduction: Anaemia is a global health problem affecting both developing and developed countries with major consequences for human health as well as social and economical development. Iron deficiency anaemia is the most common nutritional anaemia in the world.The objective of the study is to determining prevalence and risk factors contributing to iron deficiency anaemia between 6 months to 5 years age group children admitted in our hospital.

Aims & Objectives: 1.To study prevalence of iron deficiency anaemia. 2. To study factors affecting iron deficiency anaemia
Materials & Methods: This was a hospital based observational cross sectional study of 100 children admitted at the pediatric department, Midnapore Medical College between 6 months to 5 years of age. The study was done with the help of a pretested questionnaire, clinical examination and blood parameters including hemoglobin,serum iron profile to confirm the diagnosis.
Result: The prevalence of iron deficiency anemia was 65% among the study population. 81% low birth weight babies were affected with iron deficiency anemia compared to those whose birth weight were normal (more than equal 2.5 kg) p value 0.00279 which was significant. Low serum ferritin was found in 92% patient with iron deficiency anemia. 81% were suffering from iron deficiency anemia whose complementary feeding started late p value is 0.0237 which was statistically significant. Low socioeconomic status, overcrowding, malnutrition and those who started cow milk at an early age were also found to be risk factors in our study which was statistically significant. Those who received albendazole for deworming after 2 years of age have less prevalence of iron deficiency anemia which was statistically significant. 40% children having iron deficiency anemia were taking facilities of the National Iron Plus Initiative and 80% having anemia in children who did not receive iron supplements under the National Iron Plus Initiative, which was statistically significant.
Conclusions: Prevalence of iron deficiency anemia remains a major health problem in our country. Low socioeconomic status plays a major role in it and serum ferritin is the most sensitive marker of iron deficiency anaemia helps diagnose it early in its course.

Prevalence of Double Heterozygous Haemoglobinopathies in Odisha: a 5 Year Study in a Tertiary Care Centre

Ritu Priya Choudhary, Raka Hota, Rajesh bhola, Sarita Pradhan, Priyanka Samal, Debahuti Mohapatra

Introduction: Hemoglobinopathies are hereditary disorders arising from changes in the amino acid sequence and a decrease in globin chain production which affect the structure, function or production of hemoglobin. Common hemoglobinopathies in India include thalassemia, along with HbS, HbE, and HbD and their combinations. Hemoglobinopathies can occur in the heterozygous and homozygous states. A change in amino acid sequence in both α and β chains, termed double heterozygosity. The double heterozygosity for α and β chain variants leads to the formation of abnormal heterodimer hybrids, which can lead to diagnostic dilemmas.
Aims & Objectives: 1. Evaluate the prevalence of double heterozygous haemoglobinopathy in tertiary care centre of eastern India. 2. Correlation of RBC parameters with capillary zone hemoglobin electrophoresis reports and family screening.
Materials & Methods: A nearly 5 yrs retrospective study was performed from January 2018 to August 2022 in the Dept of Lab Hematology in IMS and SUM Hospital, Bhubaneswar, Odisha. The capillary zone Hemoglobin electrophoresis data were evaluated. Hematological parameters of these patients were also analyzed and correlated with respective Hemoglobin Electrophoresis findings. Family screening of cases was also done wherever possible.
Result: Total 5033 cases were evaluated in the mentioned period,of which we got 49 cases which were Double Heterozygote with 27 cases of sickle- beta thalassemia, 13 cases of HbE- beta thalassemia, 7 cases of sickle- alpha thalassemia and 2 cases were sickle cell/HbD-Punjab. In Hb E-β thalassemias, significant negative correlation noted between hemoglobin and Red cell Distribution Width (RDW) and also between RDW and Red Blood Cell (RBC) count. In Hb S-β thalassemias, significant negative correlation was seen between HbA2 level and RBC count.
Conclusions: Hemoglobin electrophoresis and HPLC (High performance liquid chromatography) are the gold standards of diagnosis of different types of hemoglobinopathies and its variants, followed by molecular genetic analysis. In a state like Odisha which is a sickle cell and thalassemia belt, our study provides prevalence of double heterozygote haemoglobinopathies in eastern India and also highlights the importance of hematological parameters (RBC count, RDW, Hb) in elucidation of double heterozygous haemoglobinopathies from much commoner variants of haemoglobinopathies, particularly in under resourced areas.

Clinical and Etiological Profile of Anaemia in Geriatric Patients of a Tertiary Care Hospital of Eastern India

Swati Singla, Ruchi Sinha, Surabhi, Tarun Kumar, Shreekant Bharti, Punam Prasad Bhadani

Introduction: According to 2011 census, India has around 104 million geriatric population (> 60 year), constituting 8.6% of total population. Anaemia one of the most important public health problems in developing countries like India, is more prevalent in geriatric population. Anaemia of any degree in elderly patients is associated with significant morbidity and mortality.
Aims & Objectives: To study the clinical profile of Anaemia in elderly population and subclassify based on etiology.
Materials & Methods: An observational hospital based study which included elderly patients (> 60 years), admitted in AIIMS, Patna between May2020 to July2022. A detailed history, thorough clinical examination and relevant blood investigations including bone marrow examination and biochemical parameters were done in all patients. According to WHO, Anaemia is defined as hemoglobin levels < 13 g/dl in men and < 12 g/dl in women.
Result: Out of total 62 patients, 61%(n = 38) were male and 39%(n = 24) were females. 77%(n = 48) patients were between the age of 60 to 69 years, 20%(n = 12) patients between 70 to 79 years and 3% (n = 2)above 80 years. 63% patients had severe anaemia, 30% had moderate and 7% had mild anaemia. Majority of patients (54%; n = 34) had normocytic anaemia, 28%(n = 17)had microcytic anaemia and 18%(n = 11)had macrocytic anaemia on peripheral smear. The etiological distribution of anaemia revealed haematological disorders(45%;n = 28)as the most common cause of anaemia followed by iron deficiency in 27% (n = 17), Vitamin B12 deficiency in 14% (n = 8), anaemia of chronic disease in 6% (n = 4), metastasis in 5%(n = 3)and unexplained cause in 3% (n = 2). Commonest haematological disorder was plasma cell dyscrasia(46%) followed by myelodysplastic syndrome(30%).
Conclusions: Anaemia in geriatric population is a common problem but is usually underdiagnosed. A systematic approach to diagnosis and evaluation of anaemia in elderly population will help in better management and improve their quality of life.

Deciphering the Molecular and Clinical Spectrum of Variants in Ank1 Gene Causing Hereditary Spherocytosis in Indian Patients Using Targeted Next-Generation Sequencing

Tejashree Anil More, Rati Devendra, Rashmi Dongerdiye, Prashant Warang, Prabhakar Kedar

Introduction: Hereditary Spherocytosis (HS) is a common cause of hemolytic anemia varying from mild to severe hemolysis due to defects in red cell membrane protein genes, namely ANK1, SPTB, SPTA1, SLC4A1, and EPB42. These genes are considerably very large spanning 40–50 exons making gene-by-gene analysis costly and

laborious by conventional methods. Mutations in ANK1 gene accounts as the common cause of typical dominant HS.

Aims & Objectives: We aim to diagnose and explore the clinical and molecular spectrum of variants in the ANK1 gene causing HS in Indian patients.

Materials & Methods: In this study, we explored 32 HS patients harboring 27 ANK1 variants identified by next-generation sequencing (NGS), characteristics and spectrum of the detected ANK1 variants were analyzed in this study. We have also explore expression levels of red cell membrane ankyrin protein by flow cytometry in 20 HS patients with ANK1 gene defects.

Result: Clinically, all the HS patients showed moderate to severe transfusion-dependent hemolytic anemia, some requiring splenectomy. Based on the severity of the disease, we found 3 mild, 18 moderate, 7 moderately severe, and 4 severe HS cases. We identified 17 novel and 10 reported variants, mainly 9 frameshifts, 3 missense, 9 nonsense, and 6 splice site ANK1 variants. Frame shifts were remarkably the most common variant type seen in Indian HS patients with ANK1 gene defects followed by nonsense variants. We have also explore expression levels of red cell membrane ankyrin protein by flow cytometry in 20 HS patients with ANK1 gene defects and significant reduction in ankyrin protein expression has been found. Coinheritance of HS with several other genetic disorders such as thalassemia minor leading to severe phenotypic modifications were found in 2 cases. Nearly all of the detected variants have damaging effect on the protein stability and function revealed by in silico analysis. The possible effect of the detected variants on the protein structure was studied using the HOPE software and DynaMut tools.

Conclusions: This report mainly illustrates the molecular and phenotypic heterogeneity of ANK1 variants causing HS in Indian patients. Ankyrin-1 mutations are significant cause of loss of function in dominant HS in the Indian population. Comprehensive genetic and phenotypic evaluation assists in implementing the knowledge of genetic patterns and spectrum of ANK1 gene variants, providing molecular support for HS diagnosis.

Table 1: List of identified ANK1 variants in Indian HS patients

Patient	Systemic name	Amino acid variation	Location	Type	Domain	Zygosity	ACMG guidelines	Novel/ Reported
1.	IVS5-1C	-	Intron 5	Splicing error	MBD-ANK3	Hetero	Likely Pathogenic	Novel
2.	c.358C>T	p.Gln120Ter	Exon 5	Nonsense	MBD-ANK3	Hetero	Likely Pathogenic	Reported
3.	c.382_386del	p. Lys128PhefsTer7	Exon 5	Frameshift	MBD-ANK3	Hetero	Pathogenic	Novel
4.	c.1900-18C>A	-	Imtron 16	Splicing error	MBD-ANK	Hetero	Pathogenic	Reported
5.& 6.	c.2004delA	p. Leu669SerfsTer7	Exon 18	Frameshift	MBD-ANK20	Hetero	Likely Pathogenic	Novel
7.	c.2113dup	p.Leu672ProfsTer121	Exon 18	Frameshift	MBD-ANK20	Hetero	Pathogenic	Novel
8.	c.2103_2104insT	p.Tyr702LeufsTer91	Exon 19	Frameshift	MBD-ANK21	Hetero	Likely Pathogenic	Novel
9.	c.2167C>A	p.His723Asn	Exon 19	Missense	MBD-ANK21	Hetero	Uncertain significance	Novel
	IVS1+5C in HBB gene	-	Intron 1	-	-	Hetero	Pathogenic	Reported
10.	c.2393_2403del,	p.V798AfsTer7	Exon 22	Frameshift	MBD-ANK23	Hetero	Pathogenic	Novel
11.	c.2559-2A>G 3'SS	-	Intron 25	Splicing error	SBD-ZU5A	Hetero	Pathogenic	Reported
	c.47G>A (p.W16*) in HBB gene	-	Exon 1	-	-	Hetero	Pathogenic	Reported
12.	c.2638-2A>T	-	Intron 24	Splicing error	SBD-ZU5A	Hetero	Pathogenic	Novel
13.	c.2950C>T	p.Gln984Ter	Exon 26	Nonsense	SBD-ZU5A	Hetero	Uncertain significance	Novel
14.	c.2690+1G>A 5'SS	-	Intron 26	Splicing error	SBD-ZU5A	Hetero	Uncertain significance	Reported
15.	c.3157C>T	p.Arg1053Ter	Exon 27	Nonsense	SBD-ZU5A	Hetero	Pathogenic	Reported
16.	c.3059_3066delACAGGAGC	p.His1020ProfsTer94	Exon 27	Frameshift	SBD-ZU5A	Hetero	Likely Pathogenic	Novel

Safety, Efficacy and Cost Effectiveness of Generic Ferric Carboxy Maltose for Treating Iron Deficiency Anemia in India

Harika Padamata, Uday Yanamandra, Suman Pramanikrajan Kapoor, Ankur Ahuja, Satyaranjan Das,Tathagata Chaterjee, Naveen Aggarwal

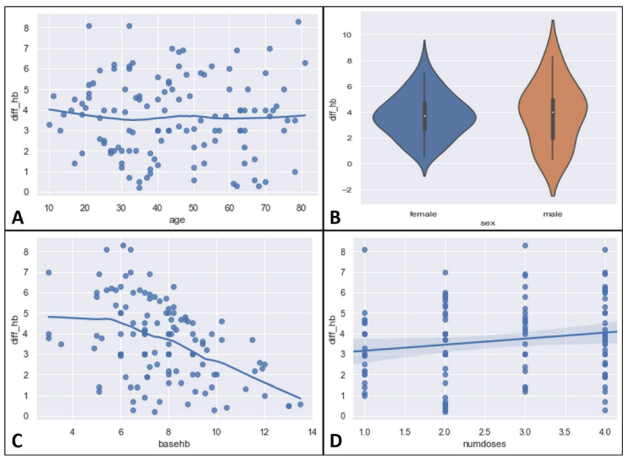
Introduction: Iron deficiency Anemia (IDA), a significant public health problem, is conventionally treated with oral iron therapy, which is encumbered by poor compliance owing to complacency or side effects. Parenteral iron is fraught with fear of infusion reactions and serious adverse events. There is a paucity of studies investigating the safety, efficacy, and cost-effectiveness of generic parenteral iron therapy, that too, in real-world non-trial settings.

Aims & Objectives: The primary outcome of the study was to assess the safety of the generic ferric carboxymaltose (FCM) in patients of IDA irrespective of the cause. The secondary outcomes were to assess the efficacy, cost-effectiveness, and determinants of the rise in hemoglobin (Hb).

Materials & Methods: This is a prospective, single-center, single-arm, open-label, interventional trial (n-123) with sequential allocation and without any masking using generic FCM (mean:1329 ± 553 mg). The side effects and efficacy were assessed until six months and at six weeks, respectively. The cost-effectiveness was considered using direct and indirect medical costs. Data were analyzed using Python-version 3.0.

Result: The mean age of the study cohort was 44.04 ± 18.1y, with 62.6% of females. Approximately 2.5% of patients reported skin issues, nausea, and Grade I anaphylactic reactions each. There was a significant improvement in Hb over six weeks (mean rise—3.64 ± 1.9 g/dL; p-0.000).On linear regression model evaluating determinants for the rise in Hb (assessed for age, gender, baseline Hb, and the number of doses of FCM), only baseline Hb (coefficient: -0.3726, $p < 0.001$) was significantly associated with the change in Hb (Fig. 1A-D). The cost per gram of Hb rise in government and corporate settings is ₹6250 ± 8485 and ₹15,093 ± 20,650.

Conclusions: Generic FCM is a safe, effective, and cost-effective therapy for managing IDA even in resource-constrained settings.



Lactate Dehydrogenase Inhibition by Kulekhara Phytocomponents: A Possible Mechanism Behind its Hematinic Activity

Raghwendra Mishra, Anusua Singh, Roshnara Mishra

Introduction: Metabolic switching is emerging as a key determinant of cell fate decision, cell proliferation and differentiation. Molecular and enzymatic regulator of metabolic switching are promising target for drug discovery and development. Lactate dehydrogenase (LDH) mediated Warburg-like switching is reported to be associated with inflammatory phenotype and proliferation of hematopoietic stem cells whereas LDH inhibition is reported to be anti-inflammatory, anti-proliferative, and facilitates the differentiation of hematopoietic stem cell to mature blood cell.

Aims & Objectives: The present study aims to assess the LDH activity in the bone marrow cells (BMC) in murine model of anemia of inflammation (AI), and to evaluate the LDH inhibitory effect of kulekhara, in connection to its hematinic activity, under in vivo and in vitro condition and docking based virtual screening of Kulekhara phytochemicals for identification of possible drug lead against LDH.

Table 1 Summary of the clinical manifestation and genetic defects identified in the study

ID	Age of presentation (In months)	Sex	Bone Marrow feature	Response to pyridoxine	Hb (g/dL)	MCV (fl)	Transfusion history	GENE	HGVS DNA/ PROTEIN	Zygosity	Inheritance
GK	2	M	Ring Sideroblast+	R	9.3	81	None	ALAS2	c.844G > T p. (Ala282Ser)	Hemi	X
MH	11	M	Ring Sideroblast++	R	7.8	63.1	Twice	ALAS2	c.508C > T p. (Arg170Cys)	Hemi	X
AB	9	F	Ring Sideroblast++	NR	6.5	63.3	Thrice	SLC25A38	c.569C > G p. (Pro190Arg)	Homo	AR
DP	2	M	Mild dysplasia, ring Sideroblast++	ND	6. 8	70.6	Once	SLC25A38	c.569C > G p. (Pro190Arg)	Homo	AR
TS	54	M	Hyperplasia with								

X, X linked; AR, Autosomal Recessive; AD, Autosomal dominant; M, male; F, Female; R, responsive to pyridoxine; NR, non responsive to pyridoxine; ND, Not determined; MCV normal range, 80-100 fl; Hb normal range male, 12-15 g/dl; Female, 11-14 g/dl.

DyserythropoiesisND3.366.58–9 timesSLC25A38c.400C > T

p. (Arg134Cys)HomoARMS36FRing Sideroblast++NR5.976.2Every 40–45 daysHSPA9

ALAS2c.1388C > T

p. (Thr463Ile)

c.137_138delCA

p. (Pro46Glnfs*8)Hetero

HeteroAD

XPT1MMarked suppression of erythroid seriesND7.6105.5Once a weekHSPA9c.9766G > T

p. (Asp326Tyr)HeteroADX, X linked; AR, Autosomal Recessive; AD, Autosomal dominant; M, male; F, Female; R, responsive to pyridoxine; NR, non responsive to pyridoxine; ND, Not determined; MCV normal range, 80-100 fl; Hb normal range male, 12-15 g/dl; Female, 11-14 g/dl.

Materials & Methods: Turpentine oil induced experimental AI was developed in mouse and the hematological profile and BMC-LDH activity was measured in kulekhara herbal extract (KHE) treated and untreated group and compared with control. The inhibitory effect of KHE on LDH enzyme was also assessed under in vitro condition. *In-silico* docking based virtual screening was performed with KHE phytochemicals for identification of LDH inhibitor. The druggability and ADMET analysis of the selected pharmacophore was conducted for identification of drug-likeliness of the selected inhibitors.
Result: AI was confirmed from significant reduction of total hemoglobin concentration which was normalized in the KHE treated group ($p < 0 >$ in vitro enzyme activity study revealed a dose-dependent LDH inhibition by KHE. In silico docking analysis yield that the phytochemicals present in KHE can bind with LDH active site better than the standard LDH inhibitor, galloflavin. The best five drug lead satisfied the ADMET analysis and Lipinski's rule of five suggesting their pharmacophore property and ability of being good orally administrated drug.
Conclusions: It can be concluded that kulekhara might act through LDH inhibition and correct inflammatory condition and cure anemia. To support the notion further molecular simulation study is needed.

Clinical and Genetic Characteristics of Seven Congenital Sideroblastic Anemia Patients

Rashmi Dongerdiye, Prabhakar Kedar

Introduction: Congenital Sideroblastic Anemias (CSA) are a group of inherited and acquired bone marrow failure disorders defined by pathological iron accumulation in the erythroid precursors or mitochondria. Anemia in CSA is variable and characterized by hypochromic, microcytic with systemic iron overload. The genes responsible for causing CSA are ALAS2, SLC25A38, HSPA9, SLC19A2, TRNT1, ABCB7 and others. A remarkable number of CSA cases remains undefined genetically, but recent application of next generation sequencing (NGS) has recognized novel causes of CSA. Here, we describe seven cases of CSA identified by targeted-NGS.
Aims & Objectives: The study aims to evaluate congenital sideroblastic anemia patients. Also, to understand the clinical manifestations of the diseases and identify the genes involved in it by targeted-NGS.
Materials & Methods: Suspected cases of CSA based on bone marrow examination were recruited in the study. The libraries for t-NGS were prepared and sequenced to mean > 80-100X coverage on Illumina platform. Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases—ClinVar, OMIM, GWAS, HGMD and SwissVar. Non-synonymous variants effect was calculated using PolyPhen-2, SIFT, MutationTaster2 and LRT.
Result: The detailed clinical examination of the patients revealed microcytic anemia (in 5 of 7 cases), presence of ring sideroblasts (in 5 of 7 cases), dyserythropoiesis (in 2 of 7 cases), and iron stores in bone marrow (Table 1). Presently, only two patients are responding well to pyridoxine while others are on blood transfusion support. We report two patients with XLSA, inheriting two hemizygous mutations in gene ALAS2: p. (Ala282Ser) and p. (Arg170Cys). We have also identified two homozygous mutations in SLC25A38: p. (Pro190Arg), p. (Arg134Cys) and two heterozygous mutations in HSPA9: p.

(Asp326Tyr), p. (Thr463Ile). However, the latter is co-inherited with 2 base deletion in ALAS2 causing frameshift mutation c.137_138delCA p. (Pro46Glnfs*8). The functional consequences of the variants were assessed and its effect on the protein structure were investigated using PyMoL.

Conclusions: In view of the results obtained in this study, we suggest bone marrow examination combined with genetic sequencing offers reliable and confirmed diagnosis to the CSA patients.

Hereditary Hemolytic Anemia Due to Rare Red Cell Enzymopathies in Indian Population

Prabhakar Kedar, Rashmi Dongerdiye, Prashant Warang

Introduction: Hereditary red blood cell enzymopathies are genetic disorders affecting genes encoding red blood cell enzymes. Enzymopathies affect cellular metabolism in red cell which mainly consist of anaerobic glycolysis, the hexose mono phosphate shunt pathway, glutathione metabolism and nucleotide metabolism. The disease is mostly transmitted in autosomal recessive trait and X-linked in few enzymes deficiency. Enzymopathies are commonly associated with normocytic normochromic haemolytic anaemia. Diagnosis is based on detection of reduced specific enzyme activity and molecular characterization of the defect on the DNA level. However, the intervention of transfused blood, incomplete removal of leukocytes and platelets, retic count can give false results.

Aims & Objectives: The study aims to identify the different red cell enzymopathy, molecular status and impact of novel variants on disease severity.

Materials & Methods: The enzyme activity was measured using conventional spectrophotometer-based assay, followed by final confirmation by t-Next Generation sequencing and DNA Sanger sequencing. The genotype–phenotype correlations of novel variants were established by bioinformatics prediction tools and validated by functional study.

Result: The clinical symptoms observed in our patient cohort varies from mild to fully compensated hemolytic crisis necessitating neonatal exchange transfusions and/or subsequent regular transfusion support; complications include gallstones, iron overload, mental retardation, developmental delay, rhabdomyolysis. five enzymopathies of anaerobic glycolytic pathway i.e. pyruvate kinase deficiency (45), glucose phosphate isomerase deficiency(21), hexokinase deficiency(2), aldolase deficiency (1), phosphoglycerate kinase deficiency (1) and two of nucleotide metabolism—adenylate kinase deficiency (3) and pyrimidine 5' nucleotidase deficiency(5)has been identified in the study.

The functional consequences of the variants were assessed and its effect on the protein structure were investigated using bioinformatics prediction tools. ACMG guidelines are used to label the novel variants as variants of uncertain significance (VOUS), or pathogenic.

Conclusions: The possibility of rare red cell enzymopathies in these cases with hemolytic anemia and some cases with neurological disorder in the absence of acquired autoimmune process, hemoglobinopathy and membranopathy should always be considered. This study examines the extensive molecular heterogeneity of red cell enzymopathies, focusing on the diagnostic impact of genotypes and new acquisitions on pathogenic non-canonical variants. Timely determination is helpful in diagnosis, genetic counselling, and offering a prenatal diagnosis.

Table1- Glimpse of red cell enzymopathies identified by t-NGS in the study

Sr. No	Gene	No. of cases	Clinical manifestation	Inheritance	Disease
1.	PKLR	45	Haemolytic Anaemia	AR	Pyruvate kinase deficiency
2.	GPI	21	Haemolytic Anaemia ± Mental retardation,± Developmental delay	AR	Glucose phosphate isomerase deficiency
3.	NT5C3A	5	Haemolytic Anaemia	AR	Pyrimidine 5' nucleotides deficiency
4.	AK1	3	Haemolytic Anaemia ± Mental retardation ±Developmental delay	AR	Adenylate kinase deficiency
5.	HK1	2	Haemolytic Anaemia ±Developmental delay	AR	Hexokinase deficiency
6.	PGK1	1	Haemolytic Anaemia ± Mental retardation	X-linked	Phosphoglycerate Kinase deficiency
7.	ALDOA	1	Haemolytic Anaemia ±Developmental delay	AR	Glycogen storage disease.

Serum Holotranscobalamin and Methylmalonic Acid as Early Markers of Vitamin B12 Deficiency in Pregnant Females

Fallguni Arora, Sunita Sharma, Kiran Aggarwal

Introduction: Vitamin B12 deficiency occurs frequently, especially among the vegetarians. Screening for vitamin B12 deficiency in pregnancy is not routinely done in antenatal care. Serum vitamin b12 assay has low sensitivity and specificity, therefore, this study was conducted to assess the usefulness of holotranscobalamin (holoTC) and methylmalonic acid (MMA) as an early marker of functional vitamin B12 deficiency in pregnant females.

Aims & Objectives: To estimate the levels of holoTC and MMA in pregnant females and correlate the levels of serum holoTC and MMA with levels of serum cobalamin (cbl) in pregnant females.

Materials & Methods: Hundred blood samples were collected from first trimester pregnant females. Vitamin B12 was measured by chemiluminescence method and serum holoTC and MMA were measured by enzyme immunoassay.

Result: The serum cbl levels were reduced (< 200 pg >

Conclusions: HoloTC could be a better diagnostic marker than vitamin B12 to detect subclinical deficiency. Although a sample size of 100 cases was taken but normal cbl level was observed only in 10 cases, out of which 9 had low holoTC, but it was a very small group to make a conclusion.

Hemoglobin Reims: A Rare Alpha Globin Chain Variant Eluting in Hbs Window in Hplc and its Interaction with Beta Thalassemia

Millu Jain, Amar Dasgupta, Trupti Shetty, Leena Salunkhe, Manju Goriwale, Anita Nadkarni

Introduction: High performance liquid chromatography (HPLC) is widely used as the primary method of screening for hemoglobinopathies. Non-sickle hemoglobin (Hb) variants that elute in HbS window in HPLC pose diagnostic challenges, especially in HbS prevalent geographies.

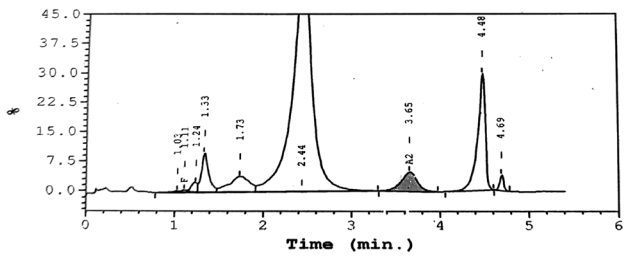
Aims & Objectives: We describe here two brothers (Patients 1 and 2) with Hb Reims, a rare alpha globin chain variant that eluted in HbS window and highlight the interaction of this hemoglobinopathy with beta thalassemia that was present in one of the two brothers.

Materials & Methods: Hb analysis was performed by HPLC. Covalent reverse dot blot and refractory mutation system (ARMS) were used for detection of common beta globin gene mutations. Alpha and beta globin gene mutation analysis was performed by DNA sequencing.

Result: Both brothers were clinically asymptomatic and had 'thalassemia trait-like' red cell indices. HbA2 was high (4.9%) in patient 2 and normal (2.7%) in patient 1. HbF was normal (0.3%) in both. The abnormal Hb peaks in patient 1 (21.7%) and patient 2 (13.8%) eluted at 4.51 and 4.48 min. respectively in HbS window in HPLC. Sickling test was negative in both. Gene sequencing confirmed heterozygous Hb Reims in both resulting from an HBA1:c.71A◇C, Glu◇Gly; Codon 23 (GAG◇GGG) mutation of alpha 1 globin gene.

Both also had an alpha globin gene deletion (-α3.7/αα). Patient 2 additionally had heterozygous beta thalassemia resulting from Codon 15 (G◇A) beta globin gene mutation. The brothers showed a very small abnormal Hb peak (retention time 4.69 min) immediately following Hb Reims peak possibly representing 'Hb A2 Reims' resulting from the combination of the abnormal alpha globin chain of Hb Reims with the normal beta chain.

Conclusions: Hb Reims is a non-pathogenic Hb variant that needs to be distinguished from HbS. A co-existent beta thalassemia seems to have lowered the level of Hb Reims in patient 2. Both patients had a small 'HbA2 Reims' peak resulting from combination of abnormal alpha globin chain of Hb Reims with normal beta chain. Only one case of Hb Reims has been reported earlier in the world literature and none from India.



Refractory Nutritional Anaemias: A Prospective Clinical Study

P K Sasidharan, Maria Davis

Introduction: Anaemia continues to be one of the major public health concerns in India. Nutritional anaemias are due to iron, B12 and folic acid deficiencies. Identifying one deficiency alone is not enough in managing anemias. Correct diagnosis and early detection of nutritional anaemia can be done by studying the dietary habits and clinical features of patients. Lack of balanced diet is the root cause of all nutritional deficiencies including nutritional anaemias. With proper dietary practices most of the common anaemia related problems can be controlled.

Aims & Objectives: To study the clinical profile of patients referred as refractory nutritional anaemias, to identify the cause of refractoriness, to create awareness about the treatable causes and to create awareness about importance of balanced diet, along with the haematinics, in the management of anaemia.

Materials & Methods: This was a prospective cohort study, conducted on 50 consecutive patients referred as refractory nutritional anaemias. The patients were evaluated thoroughly regarding their symptoms, physical signs, dietary habits and had done necessary laboratory tests. They were prescribed hematinic including B12, folic acid and iron and were advised to follow a balanced diet plan. Relief of symptoms and signs and improvement in hemogram were recorded at the end of the study and results were subjected to statistical analysis.

Result: Among the 50 patients studied in 80% of them the refractoriness was due to combined deficiency of vitamin B12, folic, iron protein deficiency while 20% only were diagnosed with true refractory anaemia. Nutritional refractory anaemia was more in females and 45 to 50% of patients belonged to the middle class. Fatigue and anorexia were the most common symptoms and pallor was the most common examination finding. The subjects had a significant improvement in the symptoms, signs and hematological parameters after following a balanced diet with haematinics.

Conclusions: Cases referred as refractory anaemia is often diagnosed without considering the dietary habits and other coexisting nutritional deficiencies. Before labelling anaemias as refractory anaemia or MDS, combined nutritional deficiencies especially of proteins, B12 and folic acid deficiency with or without iron deficiency has to be excluded. Identifying the factors contributing to anaemia is possible by a combination of good dietary history, physical examination and hemogram rather than relying on any single laboratory test or investigation. B12, folic acid or iron deficiency in the subjects were due to multiple causes including drugs like metformin or PPI, lack of balanced diet and these were the reasons for refractoriness of nutritional anaemia. There was positive correlation to poor protein intake and decreased fruits and vegetable intake. Lack of awareness about balanced diet appeared to be the primary reason for not consuming a balanced diet as majority of the subjects were from middle- and upper-class. It was the Dietary history that suggested the possibility of nutritional anaemia as the cause for refractoriness and was supported by clinical features and the lab tests. Fatigue was the most common symptom present in the study participants. Mixed deficiency anaemia is the most common nutritional deficiency anaemia in this study.

Efficacy of Thalidomide in Combination with Hydroxyurea in Patients of Sickle Cell Anaemia: A Randomised Control Trial

Anindita Paul, Priyanka Samal, Rasmita Mishra, Santosh Kumar Singh, Pritish Chandra Patra, Harshwardhan Bahirat

Introduction: Hydroxyurea (HU) is a known Hemoglobin F inducer approved forSCA.Itis associated with reduced incidence of vaso-occlusive crisis (VOC), a common complication of sickle cell anemia.It has been proposed that thalidomide and its analogs, can emerge as a promising novel class of drugs for the treatment of Hemoglobinopathies like E-Beta thalassemia, Thalassemia major and SCA through their combined ability to reduce inflammation and to induce HbF production.

Aims & Objectives: To analyze the effect of combination of Thalidomide and HU in decreasing VOC and increase in haemoglobin in patients with SCA.

Materials & Methods: This is an ongoing single centre Randomized Controlled Trial in which patients between 18 to 45 years were enrolled excluding females of child bearing age.Patients were randomized to intervention and control group.Group A- Study arm: Thalidomide (50 mg/day) + HU (15 mg/kg/day) + Folic Acid (5 mg/day)Group B- Control arm: HU(15 mg/kg/day) + Folic Acid (5 mg/day) + Multivitamin.

Both groups werefollowed every 3 months and analyzed for reduction in VOC episodes, Hb increment and frequency of hospitalization.

Result: Out of 74 patients, 48(65%) were males and 26(35%) females.Mean baseline Hb in Group A was 8.13 g/dl and in Group B was 9.06 g/dl before therapy.After 3 months of therapy, Mean Hbin Group A was 10.59 gm/dl ($p < 0.05$),Group B 9.4gm/dl.After 3 months therapy,Retic count in Group A was 3.13%, group B was 3.45% Frequency of VOC was significantly low in group A($p < 0.05$) as compared to Group B after 3 months of therapy.Mean baseline HbS in Group A was 70.4% and Group B,72.1%.After 3 months,HbS level in Group A was 67.5% and Group B70%.Mean increase of HbF inGroup A was 24% ($p < 0.002$) compared to Group B (12%)($p < 0.03$).

Common toxicities observed were somnolence—grade 1 (5/38) (13%), grade I peripheral neuropathy(2/38)(5%), and constipation grade 2 (22/38) (58%).

Conclusion: Combination of Thalidomide withHydroxyureawas found to be effective in decreasing frequency of VOC and increase in

haemoglobin inpatients with SCA.However further follow up is required to estabish the efficacy.

Neonatal Screening Approaches for Sickle Cell Disease: Evaluation And Outcome

Kalpita Ganpat Gawit, Anita Nadkarni,Manisha Madkaikar, Malay Mukharjee,Sapan Borah

Introduction: There is no national neonatal screening program currently active in India. Early intervention like antibiotic prophylaxis, vaccination may reduce morbidity and mortality. This study was undertaken for an assessment of newborn screening approaches to observe the effect of early intervention in Sickle Cell Disease (SCD).
Aims & Objectives: To undertake a newborn screening program for SCD in tribal population for early detection and early intervention to measure the benefit of the early comprehensive care of SCD by studying the role of genetic modifiers for disease severity.
Materials & Methods: It is a prospective, interventional, follow up study conducted from August 2019 to August 2022 in two district hospitals of central India (Maharashtra). Newborn Cord blood samples were collected in EDTA tubes, and the diagnosis was done by HPLC (Bio-rad Variant II). After DNA confirmation and family screening, SCD newborns were enrolled for follow up every three months for clinical and haematological evaluations and are administered antibiotic prophylaxis T. Pentid V, T. Folic acid and vaccination- e.g. pneumococcal, meningitis, typhoid as per schedule.
Result: A total of 59 (0.4%) babies were identified with SCD {SS-[n = 51 (86.4%], Sβ thalassemia -n = 8 (13.5%), {M: F-29:30}, {Age range-0.1 to 3 years}. The highest prevalence was observed in the tribal (51%), especially in Gond (30.9%) > Madia (12.7%) > Pradhan (1.8%) > Mana (1.8%) > Halba (1.8%) tribes. Mahar-SC (30%), OBC-(16%) are also affected in Non-tribal population. Clinical and haematological evaluations done every three monthly. Presence of ameliorating factors such as alpha thalassemia and Xmn1 polymorphism had an effect on clinical severity. The highest clinical severity was observed in non-tribal. Acute respiratory infection, Acute Fibril illness, severe anemia were major complications which lead babies to hospitalization and blood transfusion (Table 1).Clinical presentation was reported as follows severe (27.11%), moderate (22.1%) whereas, 50.8% babies had mild presentation.
Conclusions: Hence Newborn screening with early intervention and comprehensive care will play a major role in reducing the mortality and morbidity. This will benefit the SCD community to improve the quality of life and longevity in India.

	Clinical Events	Events per person year (years of observation= 32, n=24)
1	Hospitalizations	0.49
2	Painful events	0.03
3	Blood transfusions	0.21
4	Acute Febrile illness (AFI)	1.0
5	Acute Respiratory infection (ARI)	1.52
6	Vasoocclusive crisis	0.03
7	Sepsis	0
8	Stroke	0
9	Sequestration crisis	0
10	Anemia	3.2
11	Splenomegaly	0.09
12	Death	0
13	Others(joint swelling, scabies. COVID-19, Shingles, GI infection)	0.28

Table1. Incidence of occurrence of clinical events in SCD babies.

Experience with Hydroxyurea and Thalidomide in Transfusion Dependent Thalassemia in a Resource Limited Tertiary Care Center

Revanth Boddu, Uday Yanamandra, Rajat Bahl, Kundan MIshra, Suman Pramanik

Introduction: Hematopoietic stem cell transplantation remains the definitive therapy for transfusion-dependent thalassemia (TDT). But many are ineligible owing to affordability, donor availability, and late presentation. The transplant-ineligible are heavily dependent on regular transfusions with chelation, whose cost and availability are significant concerns in resource constraint settings. Hence, drugs that are affordable and have a significant reduction in transfusion requirements are the need of the hour.
Aims & Objectives: To study the efficacy and safety of a low-cost therapy using a combination of hydroxyurea and thalidomide in transplant-ineligible TDT patients.
Materials & Methods: A total of 57 transplant-ineligible TDT patients above 4 years were enrolled in the study after mutational analysis and screening for hepatic dysfunction. The individuals were given thalidomide and hydroxyurea (dosed using weight-based nomograms) in addition to the standard of care. They were monitored quarterly for transfusion requirements and monthly for any medication-related adverse effects.
Result: The mean age of the study population was 13.8 ± 6.54y with a male preponderance (54.4%). The average transfusion requirement at baseline was 1.39/month, which was reduced to 0.96/month. The mean duration between transfusions increased from 22.5 ± 4.6 to 50.3 ± 4.4 days. Post 200 days of therapy, 40% of patients were transfusion free with the persistence of benefit during follow-up. None of the patients had any severe drug-related adverse events requiring discontinuation. There was no clear correlation of the transfusion benefit with any specific mutations, IVS I-5 (G > C) being the most common (66.67%).
Conclusions: Using a combination of hydroxyurea and thalidomide was safe and effective in reducing the frequency of transfusion across age groups.

Hematological and Genetic Profiles of Persons with Co-Inherited Heterozygous Beta-Thalassemia and Supernumerary Alpha-Globin Genes

Durgadevi Sundaresan, Prashant Sharma, Reena Das, Jasbir Kaur Hira, Sanjeev Chhabra1, Amita Trehan, Alka Rani Khadwal

Introduction: Thalassemias are common monogenic autosomal recessive hemoglobin disorders. The usually asymptomatic heterozygotes (β-thalassemia traits, βTT) may rarely develop non-transfusion-dependent-thalassemia (NTDT) due to co-inheritance of supernumerary α-globin genes. Literature on phenotypic/genotypic features of these rare combinations is limited.
Aims & Objectives: To assess the hematological, genetic and clinical profiles of such persons with a view to guide clinical suspicion and laboratory testing practices in βTT cases presenting with unusually severe clinical phenotypes.
Materials & Methods: We studied 47 persons with coinherited βTT + supernumerary α-globin genes. Demographic, clinical, and laboratory data were recorded. We performed HBB mutation testing by ARMS-PCR and/or Sanger sequencing, $\alpha\alpha\alpha^{n}$ (anti3.7) / $\alpha\alpha\alpha^{n}$ (anti4.2) and deletional α-thalassemia testing by multiplex gap-PCRs, Xmn1Gγ genotyping by PCR–RFLP and Gilbert syndrome screening by Sanger sequencing.
Result: The 47 cases comprised 0.08% of 61,010 hemoglobinopathy screenings. The mean age was 31.9 ± 14.7 years (range 5.5 to 83 years), with 57.4% males. Thirty (63.8%) had NTDT-phenotype, 16 (34%) were asymptomatic/minimally symptomatic, and one became transfusion-dependent. Anemia/pallor and jaundice were the commonest complaints (76.

Conclusions: This largest Indian and globally second-largest study reports the βTT + ααα4.2 state for the first time in such genotypically-complex Indian cases. Supernumerary α-genes should be suspected in all βTT with disproportionate clinical symptoms, mild-to-moderately elevated HbF, and unexplained anisopoikilocytosis.

High Incidence of Aplastic Anemia Among Cases of Peripheral Blood Pancytopenia in North India

Kusum Gupta, Ruchi Gupta, Naresh Tripathi, Khaliqur Rahman, Dinesh Chandra, Manish Kumar Singh, Sanjeev, Rajesh Kashyap

Introduction: Aplastic anemia (AA) has a higher incidence in Asia than in western countries, but the precise incidence of the disease in India is not yet known due to a lack of systemic epidemiological studies. Since peripheral blood pancytopenia (PCP) is an essential diagnostic criterion of AA, we evaluate the incidence of AA among cases of PCP received at our institute, which is a major tertiary care centre in North India.
Aims & Objectives: The study aimed at analysing the true incidence and epidemiological features of AA amongst the cases presenting with PCP.
Materials & Methods: We conducted a retrospective study of all patients, presenting with pancytopenia(PCP) and concurrently going bone marrow (BM) examinations from August 2020 to August 2022. The patient details were extracted from electronic medical records. The diagnosis and severity of AA were established according to Camitta's criteria.
Result: A total of 803 cases of PCP underwent BM examination. Of these, 320 (39.9%) were diagnosed as AA, 253 (31.5%) as Acute leukemia, 56 (7.0%) as Myelodysplastic syndrome, 130 (16.2%) as Normal marrow (cellular marrow with tri-lineage hematopoiesis), 18 (2.2%) as Non-Hodgkin's lymphoma, 7 (0.9%) as metastasis, 5 (0.6%) as hemophagocytic lymphohistiocytosis, 9 (1.1%) as leishmania and histoplasma infections, and 5 (0.6%) as nutritional deficiency anaemia. The median age of the AA patients was 25 years (range: 15 to 55 years) and the male/female ratio was 2.1:1. A total of 79 (31.2%) AA patients were of pediatrics (< 14 years). The hematological parameters of the AA patients showed a median haemoglobin level of 6.7 gm/dL, a white blood count of 3,600 cells/mm3, an absolute neutrophil count of 700 cells/mm3 and a platelet count of < 20,000/mm3. The evaluation of paroxysmal nocturnal hemoglobinuria (PNH) in 230 (71.9%) of AA patients by flow cytometry showed PNH positivity in 127 (55.6%) of the patients. Of these PNH positive patients, 12.2% and 43.5% were pediatric and adult patients, respectively. The median PNH clone size was 0.33% in polymorphs and 0.48% in monocytes (minor clones). Most of the observed AA patients belonged to rural areas and had low socioeconomic status.
Conclusions: Our data shows a high incidence of AA among cases of PCP, and approximately one third of AA patients belong to the pediatric age group. The rural living and low socioeconomic status of the patients point towards the role of malnutrition and environmental factors in the etiopathology of AA.

Haematological Indices Study to Differentiate Beta Thalassemia Trait and Iron Deficiency Anemia

Jyoti Sharma, Gurpreet Kaur, Arijit Sen

Introduction: The most common hematological disease is anemia resulting from insufficient iron to synthesize hemoglobin. It has been estimated that30% of the global population suffers from iron deficiency anemia (IDA), and most of those affected live in developing countries. Microcytic anemia in the case of thalassemia results from impaired globin chain synthesis and decreased hemoglobin (Hb) synthesis, resulting in microcytosis and hypochromia; 1.5%of the world's population carries genes for.
Aims & Objectives: To differentiate 12 indices to distinguish.
Materials & Methods: A total of 164 carefully selected patients with anemia aged 6 months–73 years were evaluated. We calculated 12 discrimination indices in all patients with hemoglobin (Hb) values of 8.7–11.4 g/dL. We calculated 12 discrimination indices in all patients with hemoglobin (Hb) values of 8.7–11.4 g/dL. None of the subjects had a combined case of IDA and.
Result: The Mentzer index was the most reliable index, as it had the highest sensitivity (80.7%), specificity (90.2%), and Youden's index (70%) for detecting.
Conclusions: The Mentzer index provided the highest reliabilities for differentiating.

Evans Syndrome: A Case Report

Santosh Kumar, Avinash Kr.Singh, Divya Krishna, Ankit Kumar, Shantanu Kumar, Khursid Mallick, Sayed Shamsher Ahmad

Introduction: Evans syndrome is defined as the concomitant or sequential association of warm autoimmune hemolytic anaemia with immune thrombocytopenia, and less frequently autoimmune neutropenia. It is associated with non-cross-reacting auto-antibodies directed against antigens specific to red blood cells, platelets or neutrophils. Clinical symptoms could be related to hemolysis and thrombocytopenia. Evans syndrome is a rare diagnosis of exclusion. The first-line treatment of Evans syndrome is intravenous corticosteroids or intravenous immunoglobulins and second-line treatment with rituximab or splenectomy for those who are refractory to steroids.
Aims & Objectives: To report of rare disease which we missed in routine clinical practice.
Materials & Methods: Hospital based case report.
Result: Here is a case of a 3 year-old- female who presented with bleeding from the mouth and gums, subconjuctival hemmorrhage bluish patches over the shin and trunk along with generalised weakness and fever. Initially she was diagnosed as ITP, RECEIVED IVIG and steroid outside, post IVIG there is no improvement of thrombocytopenia, evaluated in our center,BMA/BX done outside reviwed s/o of ITP then we have started Romiplostim sc, but again no improvement of thrombocytopenia even 2 dose of romiplostim. After 2 wk presented in ER with subconjuctival bleeding, evaluated clinically she had splenomegaly, and low Hb and thrombocytopenia again re-BMA/BX was done which showed, normal megakaryocytic bone marrow, s/o evans syndrome.
Conclusions: We are interested in reporting this case because the presentation of patients with such scenarios on our part will compel the treating physician to overlook Evans syndrome and get it under diagnosed.

Bleeding Disorders

Flow Cytometric Platelet Micro-Aggregation Testing Reveals Altered Platelet Function in Bleeding Patients with Low Platelet Counts

Harpreet Virk, Man Updesh Singh Sachdeva, Narender Kumar, Praveen Sharma, Sunil Bose, Pankaj Malhotra, Jasmina Ahluwalia

Introduction: Variations in the bleeding tendencies at a comparable platelet count calls for their functional assessment. Recently developed flowcytometry- based assessment of in-vitro platelet micro-aggregation has proven to be an effective method in hematological

disorders having a functional defect and risk of clinically significant bleeding.

Aims & Objectives: To assess the platelet aggregation defects by flow cytometry based microaggregation test in thrombocytopenic patients (immune thrombocytopenia (ITP), bone marrow hypoplasia and acute leukemia) and to correlate the results with their bleeding phenotype.

Materials & Methods: We evaluated 25 thrombocytopenic and 25 normal controls for defects in response to 5 agonists (ADP, Ristocetin, Arachidonic acid, Collagen and Epinephrine). Platelet function defects (PFD) were demonstrated either in the form of alteration in the scatter properties [mean value (MFI) of Forward Scatter (FSC) channel] of the platelet aggregates or altered micro-aggregation by measuring % double coloured (

Result: Overall all thrombocytopenic patients had significantly increased MFI FSC compared to normal controls in the un-stimulated and post agonist-stimulated state. In the unstimulated state, 68% of cases had defective aggregation versus controls suggesting a possible functional defect. Post stimulation, defective aggregation with any one of the five agonists was demonstrated in 24/25 (96%) patients and mostly encountered with ADP (80%) and Ristocetin (68%). All patients who had a positive bleeding history prior to the evaluation had an increased MFI FSC of un-stimulated platelets compared to non-bleeders. At comparable platelet counts, the median % aggregation at baseline and post- stimulation was lower for bleeders compared to non-bleeders suggesting a functional defect. Based on ROC curve analysis, seven significant cut-off values were found which successfully predicted bleeding in all cases (100%) on follow up. Based on ROC curve analysis, seven significant cut-off values were found which successfully predicted bleeding in all cases (100%) on follow up which earlier had no bleeding. (Table 1).

Conclusions: Flow-cytometric platelet micro-aggregation testing identified PFD in 96% of thrombocytopenic patients and predicted bleeding in 100% of "non-bleeders" who eventually had bleeding manifestations on follow-up.

Validation of the Hemophilia Treatment Experience Measure (Hemo-Tem): A New Haemophilia-Specific Patient-Reported Outcome Measure

Rinz Paulose, Meryl Brod, Donald M. Bushnell, Jesper Skov Neergaard, Anne Kirstine Busk

Introduction: The Hemo-TEM is a patient-reported outcome (PRO) measure developed based on U.S. Food and Drug Administration (FDA) guidance to assess the burden of treatment on people with haemophilia. The measure is currently being administered in ongoing concizumab phase 3 trials.

Aims & Objectives: To evaluate and validate the psychometric properties of the Hemo-TEM for adolescents and adults.

Materials & Methods: Patients from 3 clinical trials (NN7170-4213, NN7415-4255, NN7415-4310) currently taking an injection for haemophilia (n = 88) completed a validation battery (demographics and PRO measures needed for the cross-sectional validation analysis) at a screening visit, at baseline (retest), and at 24 weeks post-baseline (n = 56, sensitivity to change). Psychometric testing, including of the measurement model, reliability, validity, sensitivity to change, and meaningful change, followed FDA guidelines for PRO measure validation.

Result: Item Reduction dropped 4 items resulting in a final 26-item measure (Figure 1). Factor analysis generated 5 domains in the Hemo-TEM [injection difficulties (3 items), physical impact (6 items), treatment bother (7 items), interference with daily life (4 items), and emotional impact (6 items)] and a total score. All scores were reliable [internally consistent (0.84 to 0.88) and reproducible (0.80 to 0.92)]. A-priori hypothesized associations for validity of the Hemo-TEM domains were confirmed. Preliminary estimates of sensitivity to change were seen with effect sizes between -0.30 and -0.70. The meaningful change thresholds ranged from 6 points (physical impact and emotional impact) to 10 points (treatment bother) with 8 points for the Hemo-TEM total score. The measure took approximately 5 min to complete suggesting minimal administration burden.

Conclusions: The Hemo-TEM can be considered a well-designed, valid, and reliable measure of the burden of haemophilia treatment on patients. This measure should prove useful to assess impacts related to haemophilia as well as to clinicians in tailoring treatments to patient characteristics and situations.

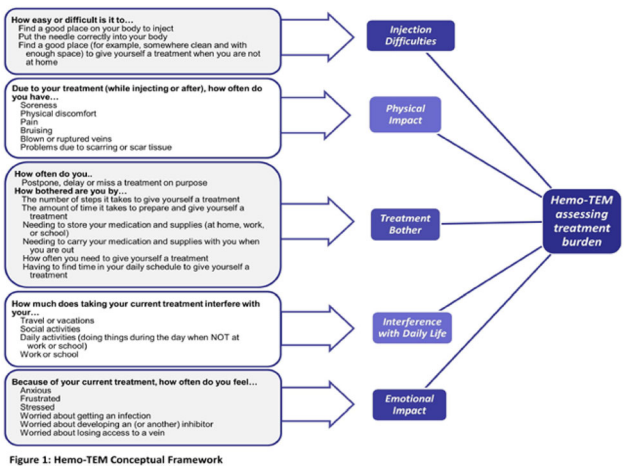


Figure 1: Hemo-TEM Conceptual Framework

Up To 6 Years of Once-Weekly Prophylactic Dosing Of Nonacog Beta Pegol (N9-GP) was Associated With High Adherence, with a Low Incidence of Spontaneous Bleeds

Ranjan KM, Anthony K. Chan, Chris Barnes, Ampaiwan Chuansumrit, Pernille Højlund Nielsen, Helle Holst, Guy Young

Introduction: Nonacog beta pegol (N9-GP) is a recombinant factor IX (FIX) with an extended half-life that minimizes treatment burden by reducing dosing frequency while maintaining high FIX trough levels with once-weekly dosing.

Aims & Objectives: To report updated data from the ongoing main and extension phases of paradigm6 (NCT02141074), an open-label phase III trial evaluating N9-GP prophylaxis in previously untreated children with haemophilia B.

Materials & Methods: The study included previously untreated males aged < 6 >

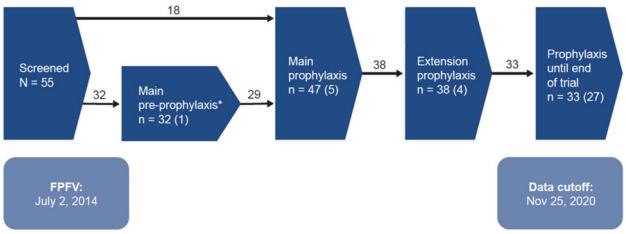
Result: Figure 1 shows numbers of patients at each trial stage. The inhibitor incidence rate was 8%. Adherence to N9-GP prophylaxis was high (96.1%). The overall haemostatic success rate was high (96.4%). Table 1 summarizes further key efficacy outcomes for patients on N9-GP prophylaxis. Notably, both overall and spontaneous ABRs were low (medians of 0.25 and 0.00, respectively), and most bleeds (88.3%) required only one injection. Furthermore, mean steady-state FIX trough levels (15.6 IU/dL) were within the range of mild haemophilia; no patients developed target joints.

Conclusions: This updated analysis provides additional data on the long-term (up to 6.11 years) outcomes following N9-GP prophylaxis. The low inhibitor incidence, favourable ABRs for prophylactic treatment, no target joint development, and high adherence demonstrate the continued efficacy of once-weekly N9-GP in children with haemophilia B.

Imatinib Induced Platelet Dysfunction: A Case Report

Alwyn Alec Lasrado, Steve Thomas

Introduction: 67 year old male from Cheyyar was diagnosed with CML—Chronic Phase (July 2021) and was treated with Imatinib 400 mg OD. H/O RTA with another 2 wheeler in Feb 2022 and was brought to the ER in view of head injury with altered sensorium.
Clinical details
Vitals Stable
CVS/RS/PA WNL
CNS—Drowsy, Arousable Obeys commands.
GCS 14/15
PERL Right periorbital edema.
Aims & Objectives: Laboratory investigations.
CBC, LFT, RFT, Urine Routine and Peripheral Smear were within normal range.
PT 12.3/PTT 22.5/INR 1.06
Course of illness: CT Brain done showed left frontoparietal moderate sized acute SDH. Mild mass effect. No midline shift. Patient was admitted managed conservatively. Repeat Neuroimaging showing no increase. Patient was discharged and advised to review after 2 weeks. Patient came back after 2 weeks with complaints of focal neurological deficits and altered sensorium. Neuroimaging done showed increase in size of bleed with midline shift. Patient was taken up for evacuation of subacute SDH (March 2022). Patient came back in April with complaints of new focal deficits. Neuroimaging suggested a new onset Subdural Haemorrhage.
Materials & Methods: Management
Hematology opinion suggested he undergo a Platelet function test that showed severe impairment in platelet function.Diagnosis of Imatinib induced platelet dysfunction was made. Patient was started on Nilotinib 300 mg BD. Repeat Platelet function test done showed normalisation. No new bleed on follow up.
Result: Diagnosis
Imatinib Induced Platelet Dysfunction with Subdural Hemorrhage in CML—CP.
Discussion: The mechanism whereby imatinib induces platelet dysfunction is not known. The activity of imatinib, or any of its pharmacologically active metabolites, on cyclooxygenase-1 is unknown. Imatinib may also alter platelet aggregation by inhibiting key kinases in platelet homeostasis. SFKs, LYN and FYN play critical roles in early platelet activation by GPVI, upstream of SYK and PLCγ2,13,14 and by integrin α2β1. Bosutinib/nilotinib, have not shown to cause any disturbance of platelet aggregation.
Conclusions: REFERENCES: www.ncbi.nlm.nih.gov/pmc/articles/PMC7900367/ A Pilot Study on Imatinib Induced Platelet Dysfunction in Patients with CML-CP.
https://pubmed.ncbi.nlm.nih.gov/31310595/ Imatinib-induced platelet dysfunction in CML—Case Report.



*An optional pre-prophylaxis period was offered (in the main phase) where patients received on-demand treatment of bleeding episodes and/or had a slow start-up of prophylaxis at intervals longer than a week until the patient had reached 24 months of age or 20 EDs, after which they received once-weekly prophylaxis for the remaining trial period.

ED, exposure days; FPFV, first patient first visit.

Figure 1. paradigm6 trial phases and numbers of patients at each phase (data cutoff Nov 25, 2020). The trial comprises a main phase* (1–3 years' duration, up to 50 EDs), an extension phase (1 years' duration, up to 50 EDs), and a prophylaxis phase until end of trial. Numbers in parentheses denote patients ongoing in the trial at time of analysis.

Efficacy and Safety of Rituximab as for Eradication of Inhibitors in Congenital Hemophilia A

Shipla Roy, Prakas Kumar Mandal, Tuphan Kanti Dolai, Rajib De, Shubhra Neel Baul,Shubham Bhattacharya, Kaustav Ghosh, Apurba Banerjee, Abhishek Maurya, Chirasree Sanyal

Introduction: Recombinant fVIII infusion used as standard treatment for acute bleeding events (on-demand) and to prevent any further bleed (prophylaxis) in patients with severe HemophiliaA(HA). The most significant treatment complication is development of neutralizing antibodies (inhibitors). Bypassing agents such as recombinant factorVII and factor eight inhibitor bypass agents(FEIBA) are effective but costly options for treatment of bleeding events. Rituximab is a chimeric monoclonal antibody against CD20 antigen on the surface of B-lymphocytes, leading to rapid and sustained elimination of inhibitors.
Aims & Objectives: Assess the outcome in patients of congenital HA patients with inhibitors treated with Inj Rituximab.
Materials & Methods: A prospective interventional study in which 16 patients of congenital HA patients with.
inhibitors attending OPD, IPD and day care of department of Haematology, NRSMCH were studied during July,2021–August,2022. Inj Rituximab@375 mg/m2 was given weekly for four weeks. FVIII inhibitor levels were studied after one week, three months and six months after the 4th dose of rituximab. Outcome was assessed in terms of the change of inhibitor level as well as incidence of annual bleed rate.
Result: Median age of study population was 11 years (2–60 years). Ten patients had severe HA. All 16 patients completed four weeks of Inj rituximab therapy followed by six months of follow-up. Seven patients had reduced annual bleed rate by 60% and six patients had reduced inhibitor level after four weeks of Inj rituximab therapy out of which two patients had zero inhibitor level titre. Three patients had increased bleeding symptoms out of which one patient is on Immune tolerance induction therapy and two are on on-demand FEIBA therapy.
Conclusion: Four weeks of Inj rituximab therapy is effective in reducing bleeding symptoms and inhibitor titres in patients with congenital HA (Table 1).

Table 1 Defective parameters fulfilling the cut-off values devised by ROC curve analysis to predict bleeding in non-bleeders with details of follow up bleeding

Case no	Defective parameters	Follow up bleeding	Follow up time period	Outcome
1	6/8	NA	NA	NA
2	8/8	Epistaxis and hematuria	1 month	Died
3	5/8	NA	NA	NA
4	8/8	Ecchymosis and easy bruisability	1 year	Alive
5	7/8	NA	NA	NA
6	6/8	Epistaxis and hematuria	4 Months	Died
7	8/8	NA	NA	NA
8	7/8	Epistaxis	1 year	Alive
9	8/8	Epistaxis	1 months	Died
10	7/8	Epistaxis, oral cavity bleeds	4 months	Died
11	4/8	No	1 year	Alive
12	5/8	No	1 week	Died
13	5/8	Echhymosis	1 week	Died

Clinico-Demographic Profile and Joint Health Score of Children with Hemophilia: Data from a Tertiary Health Center in North India

Alka Yadav, Neha

Introduction: Introduction: Clinical and joint health status determines the quality of life of hemophilic children. This study was done to assess the same at a North Indian tertiary care hospital.
Aims & Objectives: Aims & Objectives: We studied the clinical and demographic profile of patients with hemophilia aged 1–14 years and assessed their joint health by using HJHS 2.1 score.
Materials & Methods: Materials & Methods: This was a cross-sectional study done on 147 children aged 1–14 years living with hemophilia. Proper history, socio-demographic assessment, and physical examination were done as per the pre-structured performa. Assessment of joints was done by the HJHS2.1 scoring method.
Result: Results: Out of the 147 patients, 117 patients had hemophilia A and 30 patients had hemophilia B. A total of 138 (93.8%) patients had severe factor deficiency. Twenty-two (14.9%) patients had a positive family history. Around 2/3rd i.e. (66.7%) of children belong to the urban background and 76.4
Conclusions: Conclusions: A comprehensive clinical and joint health assessment using HJHS can be a reliable, quick, and effective option for the consistent assessment of joints and optimizing the management of patients with hemophilia in developing countries like India.

Delayed Hypofibrinogenemia in Saw-Scaled Viper Envenomation: A Retrospective Study from Jodhpur, India

Akhilesh Kumar P H, Maya Gopalakrishnan, Gopal Krishana Bohra, Abhishek Purohit, Bharat Choudhary, MK Garg

Introduction: Viper envenoming is usually hemo-vasculotoxic causing clinical bleeding. Cause of bleeding ranges from thrombocytopenia to life-threatening venom induced consumption coagulopathy (VICC). VICC usually responds to antivenom however, saw-scaled viper bite in Rajasthan (Echis carinatus species) has been documented to respond less to antivenoms leading to various complications due to delayed recovery. The pattern of delayed recovery of coagulation parameters or its clinical significance is unclear.
Aims & Objectives: This study describes the clinical and laboratory features of delayed hypofibrinogenemia in the background of VICC in saw-scaled viper envenomation in Western Rajasthan.
Materials & Methods: We retrospectively collected data of all saw-scaled viper envenomation admitted from January 1, 2021, to July 31, 2022, in our center using hospital records after obtaining Institute Ethics Committee approval. Clinical and laboratory details were collected. All cases of VICC were analyzed for any possible pattern of involvement.
Result: Out of 32 patients with saw-scaled viper bite, 23 patients had VICC. Among them, seven patients were not included, 5 patients who had lack of follow up fibrinogen values and two patients who had early recovery of fibrinogen. Among the 23 patients of VICC, sixteen patients had delayed hypofibrinogenemia lasting > 7 days and 7 patients lasting > 14 days. Two among the sixteen had delayed clinically life-threatening bleeds when other coagulation parameters were normalized.
Conclusions: In this study of delayed hypofibrinogenemia following saw-scaled viper envenomation, we document significant delay in the recovery of serum fibrinogen, associated with clinically significant bleeding complications after all other coagulation parameters have normalized. In addition to prothrombin activators (Ecarin), snake venom metalloproteinases (SVMPs), abundant in Echis venom have direct fibrinogenolytic activity, independent of activation of plasminogen which may persistently activate the coagulation cascade. Prolonged hypofibrinogenemia has serious implications for clinicians regarding antivenom administration and duration of follow up for saw-scaled viper envenomation patients in the region.

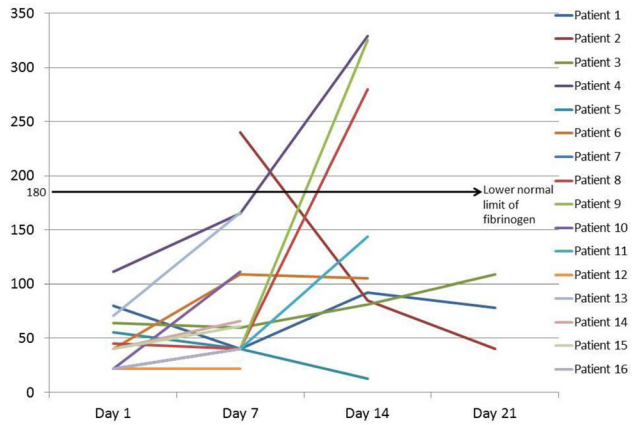


Fig 1: Trend of serum fibrinogen (normal is 180-350 mg/dl) with time in Echis envenomation

Ultrasound For Early Detection of Subclinical Bleeds in Patients with Hemophilia Receiving Prophylaxis

Moupali Ghosh, Maitreyee Bhattacharyya

Introduction: Patients with hemophilia, receiving prophylaxis should be on regular monitoring of joint bleeding. Clinical evaluation only may not be sufficient for evaluation of joint bleed which may be done by imaging. Magnetic resonance imaging is the most important modality for early detection of joint bleeding. Nowadays ultrasound has emerged as one of the most useful imaging for early evaluation of bleeding in Hemophiliacs.
Aims & Objectives: The purpose of the study is detection of subclinical bleeds in patients with hemophilia receiving prophylaxis with the help of imaging and to look for the effectiveness of prophylaxis.
Materials & Methods: The study was conducted at Institute of Haematology and Transfusion Medicine, Kolkata from April 2021 to August 2022. The most frequently involved joints of the patients were evaluated for acute bleeding and annual bleeding rates were calculated. Ultrasound imaging of the most frequent involved joints was done and was correlated with the clinical features.
Result: A total 109 patients aged 1 to 38 years receiving Hemophilia prophylaxis were included in the study. Out of the study population,46.78% presented clinically without any breakthrough bleeding and 44.03% with annual bleeding rates less than two. Imaging results has shown no abnormality in 33% of patients, 20.18% with features of joint bleeding, and 64.21% patients were detected to have other abnormality. Seven percentage had imaging reports involving multiple joints. Among the patients without breakthrough bleeding, 26.41% had ultrasound features suggestive of either bleeding or other abnormality. Among patients with annual bleeding rates less than two, 10.4% showed imaging features suggestive of joint bleed, 64.56% without any abnormality and 54.16 were detected with some other abnormality.
Conclusions: Early initiation of prophylaxis, even at low dose serves as an effective measure to decrease bleeding in hemophilia patients. Ultrasound Imaging may be considered as a routine examination for early detection of changes in hemophiliacs even in clinically asymptomatic patients and patients with low annual bleeding rates.

Observational Study on Treatment and Prognosis Of COVID-19 Related Thrombocytopenia

Divya M, Margaret C, Karthikeyan A, Abhishek Ranjan, Kishore Kumar, Vikram Yellugoti, Vandana Hari

Introduction: The hematological spectrum of Covid 19 varies from a prothrombotic state to bleeding diathesis. Thrombocytopenia in COVID infection is reported to a poor prognostic marker. As it is multifactorial with variable presentation, critical treatment decisions are challenging and individually tailored.
Aims & Objectives: To study COVID 19 induced thrombocytopenia and their response to Intravenous Immunoglobulin.
Materials & Methods: Prospective observational study.
All covid positive patients presenting with thrombocytopenia (< 50,000 >

The data regarding bleeding symptoms, comorbidities, CBC, peripheral smear, coagulation profile, viral markers, LDH, CRP, S. Ferritin, RFT, LFT, Covid severity, treatment, response and follow up data was obtained from medical records and OPD visit records.
Result: Total of 19 cases with a mean age of 50.6 years and M:F ratio of 0.9 were studied. Nadir platelet count was below 10,000/ul in 57.9
Conclusions: Thrombocytopenia in COVID can occur both in early and late phase. Close monitoring and timely intervention with IVIG in severe thrombocytopenia may be life saving. Severe thrombocytopenia during early active infection confers poor survival.

Coagulation Profile in Low and High Risk Pregnant Women in Third Trimester of Gestation

Pooja Saini, Sarika Singh, Sangeeta Gupta, Nidhi Verma

Introduction: Pregnancy is a hypercoagulable state with increased level of clotting factors, decreased concentration of some of the anticoagulants and impaired fibrinolysis. This prothrombotic state protects the women from fatal haemorrhage during delivery, at the same time predisposes women to thromboembolism and other haemostatic disorders during delivery & puerperium. The incidence of venous thromboembolism in pregnancy, is stated to be 0.76–1.72/1000 pregnancies, about 4–50 times higher than non- pregnant women, especially in the late pregnancy and puerperium. Certain conditions associated with pregnancy pose enhanced risk of coagulopathies such as high- risk pregnancy, due to pre-existent medical condition(s) of pregnancy, infections and some unknown factors.
Aims & Objectives: Primary and secondary objective were to study coagulation profile in high and low risk pregnant women in third trimester and postpartum phase and to correlate the risk of complications.
Materials & Methods: This prospective study included a total of 100 pregnant women (50 high & 50 low risk) conducted in Departments of Pathology and Obstetrics and Gynaecology at MAMC. Patients taking drugs that could affect coagulation profile were excluded from this study.
Result: Rate of cesarean delivery was higher in high risk group compared to low risk group.PT, APTT & TT levels did not show any significant changes during pregnancy and puerperium in both high & low risk group & were found within the normal reference range for non- pregnant state. However, significant statistical difference were found in TT levels within high and low risk groups in third trimester (p value 0.04), in high (p value 0.009) and low risk groups (p value 0.004) and in GTN subjects (p value 0.018). Fibrinogen, D- dimer, FDP levels were significantly higher in third trimester and in puerperium than the normal reference intervals of non- pregnant state. Thrombocytopenia was observed more commonly in high risk than low risk. Adverse outcome noted in single subject who presented with intrauterine fetal death.
Conclusions: Activation of blood coagulation parameters with simultaneous increase in fibrinolysis without any organ dysfunction is the feature of all normal pregnancy and as the pregnancy progresses, these changes become pronounce & normalize during the first 4 to 6 weeks postpartum.

Spectrum Of Genetic Changes Leading to Inhibitor Development Among Hemophilia a Patients

Debadrita Ray, Narender Kumar, Chander Hans, Anita Kler, Ritika Sharma, Manu Jamwal, Hari Kishan Senee, Jasbir Kaur Hira, Rozy Thakur, Pankaj Sharma, Minu Singh, Pratik Bhatia, Deepak Bansal, Arihant Jain, Jasmina Ahluwalia, Reena Das, Pankaj Malhotra

Introduction: Factor VIII gene variants may govern the risk of developing inhibitors in Hemophilia A (HA). Indian data on the genetic spectrum in patients with HA and their association with risk of inhibitors is scanty.
Aims & Objectives: We aimed to evaluate the genetic changes in Indian HA patients that are associated with the development of inhibitors.
Materials & Methods: All HA patients with inhibitors who availed coagulation-laboratory services from January-2015 till December-2021 and had their samples preserved for DNA extraction were included in this study. An equal number of severity-matched HA patients without inhibitors were also included as controls. Intron 22 and intron 1 inversions in Factor VIII gene were identified using inverse PCR. Inversion-negative patients were further assessed using NGS and MLPA.
Result: Thirty HA patients with inhibitors {high titre:25 (83.3%); low titre:5 (16.7%)} were identified. All had severe HA. Thirty severe HA patients without inhibitors were included as controls. Causative variants were identified in all patients. Overall, intron 22 inversion (65%), large deletions (15%) and nonsense variants (8.3%) were the commonest variants identified. There was no difference in genetic variants in patient with low and high titre inhibitors. A3, A2 and C2 were the most common domains involved in inversion-negative patients with inhibitors. However, there was no significant difference in domain involvement among inversion negative patients with and without inhibitors. All patients with multidomain involvement [n = 4 (6.7%)] had inhibitors. Among patients with point mutation or copy number variants (n = 18), exon 14 was the most common exon involved [n = 7 (38.9%]. Seven novel variants were identified including 3 large deletions, 1 large duplication and 2 nonsense variants in inhibitor positive patients, and one frameshift variant in inhibitor negative patient.
On univariate analysis, large deletions [OR:10.55 (1.23–90.67)] and intron 22 inversions [OR:0.07 (0.02–0.3)] were significantly associated with the presence of inhibitors (Figure 1). After adjusting for clinical risk factors, large deletion was independently associated with the presence of inhibitors [aOR:6.1 (1.41–56.3)].
Conclusions: Intron 22 inversions are the commonest variant in Indian patients with severe HA regardless of the presence or absence of inhibitors. Large deletions predispose to inhibitor development independent of clinical risk factors.

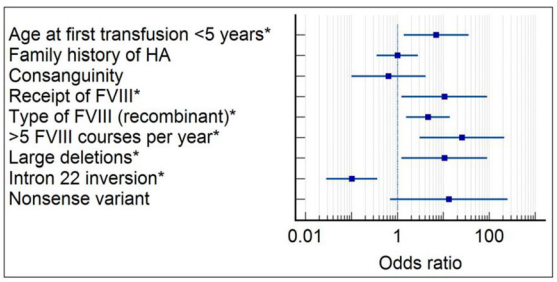


Figure 1: Odds ratios for presence of inhibitors on univariate analysis

A Retrospective Clinicopathological Study of Inherited Bleeding Disorders in a Tertiary Care Centre of Uttar Pradesh

Anu Singh, Pawan Pandey

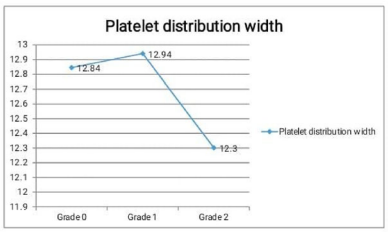
Introduction: Inherited bleeding disorders (IBD) include various diseases that reflect abnormalities of primary and secondary hemostasis. The pathophysiology of these disorders can be explained on the basis of vessel wall abnormalities, platelet disorders, and coagulation factor defects. To further elaborate and enhance our understanding of these disorders, a 6-year retrospective study (2014–2020) was conducted on the patients referred to the coagulation section of the Hematology Department (Department of Pathology, IMS BHU). These included the ones who had suffered from bleeding tendencies from one or more sites with other relevant clinical histories.
Aims & Objectives: This study aimed to assess the prevalence, clinical spectrum, and hematological profile of inherited bleeding disorder among patients of Eastern UP and Bihar. It also focuses on various epidemiological factors including age, sex, family inheritance, and consanguinity.
Materials & Methods: Three hundred and two patients matched our criteria. The age of the patients ranged from neonate to 50 years of age. A detailed relevant clinical history was taken for all the patients. This category of patients was screened with routine tests like platelet count, Prothrombin Time(PT), Active Partial Thromboplastin Time(APTT), Breathing Time(BT), Clotting Time(CT), and a Complete Blood Cell Count(CBC). A factor assay was performed if indicated by the results of the screening assays.
Result: Out of 302 patients, 280patients (92.70%) were diagnosed with factor VIII deficiency. These category further comprised of 63.57% Hemophilia A cases(n = 192), and 12.58%hemophilia B cases(n = 38). Another cluster of 16.55% was diagnosed with Von Willebrand Disease (n = 50). Also, a subset of the total patient population (7.30%) was diagnosed with an entity called Rare Inherited Coagulation Deficiency (RICD) which was further designated on the basis of specific factor assays. The most common clinical feature encountered was hematoma followed by ecchymosis, hemarthrosis, gum bleeding, and epistaxis.
Conclusions: The most common IBD was Hemophilia A in this subcategory of patients. Children under 5 years of age were most affected making it the most vulnerable age group amounting to 38.73% of all recorded cases. The male population was more affected forming the majority of the patients. Sporadic cases were more common than inherited ones.

Platelet Indices in Prognostication of Immune Thrombocytopenic Purpura

Tiji Alphonse, Michael Pushparani, Sanjukta Rao

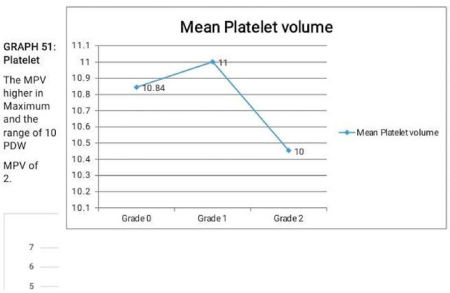
Introduction: Immune thrombocytopenic purpura is an auto-immune mediated destruction of platelets resulting in bleeding. Few studies have been done on looking at Platelet indices to prognosticate ITP. In this study we are looking at Bleeding scores and its correlation to platelet indices in ITP.
Aims & Objectives: To examine the kinetic characteristics of platelet destruction and thrombopoiesis by using mean platelet volume (MPV) and platelet distribution width (PDW) and correlating it to severity of ITP using the WHO bleeding scores.
Materials & Methods: Using the ADVIA2120i instrument, we measured PLT counts, MPV and PDW in 100 patients with immune thrombocytopenic purpura (ITP). Bleeding scores were calculated using WHO bleeding scores and its relation was studied using various statistical methods including Kruskal–wallis test.
Result: 1) The PDW values in patients with ITP were higher in grade 4 than the other grades but found to be within the normal limits. Maximum PDW noted in grade 4 which is 13.09 and the other grades 0 to grade 3 are in range of 12.Severe the disease the value of PDW

tend to be increasing, however statistically not significant. p value 0.945.
2) MPV of patients with ITP were normal in all the grades. Grade 4 with lower values than the other grades. However in all the grades the value of MPV is within the range of 10–11, with p value 0.902 not statistically significant.
Conclusions: Platelet indices- PDW and MVP did not statistically correlate to platelet counts in ITP and hence cannot be used an indicator of severity of ITP.



GRAPH 50 : Comparison of Mean of Platelet distribution width with IBLS bleeding grades:

The PDW values in patients with ITP were normal in all the grades. Maximum PDW noted in grade 1 which is 12.94 and the other grades 0 and 2 are in range of 12.3 -12.8. Severe the disease the value of PDW found to be low. The PDW values in patients with ITP were low in grade 2 than the other grades .p value 0.537 statistically not significant.



GRAPH 51: Platelet

The MPV higher in Maximum and the range of 10 PDW

MPV of 2.

Comparison of Mean of mean volume with IBLS bleeding grades:

values in patients with ITP were grade 1 than the other grades. MPV noted in grade 1 which is 11 other grades 0 to grade 2 are in .Severe the disease the value of found to be low .p value 0.552

patients withITP were low in Grade

Prevalence of Autoimmune Markers in Immune Thrombocytopenia (ITP) and its Relation to Severity

S.Michael Pushparani, Tiji Alphonse, Sanjukta Rao

Introduction: Immune thrombocytopenia (ITP) is an autoimmune disorder mediated by platelet antibodies thought to accelerate platelet destruction while inhibiting also their production, resulting in low platelet counts with potentially spontaneous bruising, petechial rash, mucosal bleeding or even life-threatening hemorrhage. Immune Thrombocytopenia is Still the Commonest Diagnosis on Consultative Hematology in India. In this context, the present study aimed at describing the clinical features of adult ITP and the biomarkers for diagnostic purposes, to know severity and the prognosis of the disease.
Aims & Objectives: To assess the prevalence of autoimmune markers in adult patients with ITP and its relation to severity of ITP.
Materials & Methods: Auto-immune profile was studied with antinuclear antibody (ANA), red cell direct antiglobulin test (DAT), antithyroid peroxidase antibodies (Anti-TPO), APLA,levels of complements C3, C4 and Immunoline.
Result: There was a high rate of autoimmune marker positivity in our study population. Of all 100 patients, 85 patients were positive for at least 1 of the markers. All the markers were positive in 2%, > 3 markers were positive in 34%, 49% were positive for < 3 markers of the tested autoimmune markers, and (15%) had negative results for all autoimmune markers tested. Of 100 patients who had all 7 autoimmune markers tested were only 2%, and 15% had no positive marker. 34% who had > 3 positive autoimmune markers were for APLA, ANA, DCT, Anti-TPO,C3,C4 and immunoline. The most prevalent positive autoimmune markers were ANA (51%), anti-TPO (48%), and APLA (41%) followed by DCT (33%), immunoline (15%),C3 (6%) and C4 (4%).
Conclusions: There was a high prevalence of auto-immune markers that were positive in the population of ITP patients studied, which suggests that many patients with ITP have a state of immune dysregulation that extends beyond platelet auto-antibodies and that certain autoimmune markers may be prognostically useful in this disorder.

Rare Presentation of Thrombocytopenia: It's Genetic or Acquired a Dilemma for Hematologist

Siyaram Didel, Varuna Vyas, Aliza Mittal, Dyvik S, Debasish Barman, Abhishek Purohit, Kuldeep Singh

Introduction: Thrombocytopenia in children can present with or without an underlying genetic cause. Although acquired causes like immune thrombocytopenia (ITP) are more common in the adolescent age group of the pediatric population. Genetic defects can present with quantitative or qualitative platelet disorders like Bernard Soulier Syndrome, Glanzmann thrombasthenia, and other syndromic conditions. Most of the time, clinicians or hematologists can differentiate between genetic and acquired causes for optimal clinical management of individual cases. Diagnosis of acquired causes of thrombocytopenia, like ITP, is a clinical diagnosis after excluding all secondary causes of thrombocytopenia. We have an atypical case of thrombocytopenia with an underlying genetic mutation with a positive family history who responded to treatment like an acquired disorder (ITP) of thrombocytopenia.
Aims & Objectives: The aim of presenting this case report is to highlight keeping a high index of suspicion for treatable acquired causes of thrombocytopenia like ITP and must give therapeutic trial if the case has any atypical clinical features even with an associated genetic mutation. while dealing with microcytic hypochromic anemia. Hence while evaluating a child with thrombocytopenia through clinical and family history is a must, along with diagnostic workup and response to therapy to confirm the diagnosis and avoid unnecessary morbidity and mortality along with wastage of resources.
Materials & Methods: The Index Case was evaluated and managed by an integrated hematology team of pediatric hematology and the Department of Pathology & Lab Medicine at All India Institute of Medical Sciences, Jodhpur. The Boy was admitted given new onset severe thrombocytopenia and active mucosal bleeding and evaluated with complete history, sequential haemogram findings, peripheral smear examination, morphology, detailed coagulation study, and bone marrow examination along with molecular genetic testing and evaluation of parents.
Result: The index case, a 14-year-old boy, presented with clinical history of multiple episodes of epistaxis which resolved spontaneously without any hospital admission. History of easy bruisibility also there. He also had history of development of rash on pinching, at site of pressure/trauma which get resolved spontaneously in 3 to 4 days. On basis of maternal history of easy bruisibility and epistaxis and maternal investigations suggestive of macro thrombocytopenia (Platelet 94,000 and MPV: 15.4) possibility of inherited macro thrombocytopenia (? BSS,? PTVWD,? MYH9? SFLN14RT, ITGA2B/B3-RT) was kept. Blood examination and hemogram were suggestive of severe thrombocytopenia with peripheral blood film showing large platelets (MPV: 15.7). Initial APPT was prolonged (46.7) hence intrinsic pathway factor (Factor VIII and IX), and VWF was sent. However, the maternal coagulogram and the patient's repeat detail coagulation study were normal. Bone marrow aspiration was done to rule out immune thrombocytopenia, which was suggestive of a mild increase in the number of megakaryocytes in marrow with normal morphology. Platelet function test was planned and was not

done because of the low platelet count. In view of bone marrow findings and atypical features in history, severe thrombocytopenia compared to mother, hence child was given a trial of immunosuppressive therapy with oral steroid and he responded well and platelet recovered to normal range. Later his genetic testing reported two mutations of uncertain significance, i.e.—hemizygous mutation of exon 15 (C.5312 T > G(p.Leu1771Arg)) with disease association Hemophilia A/Thrombophilia due to factor VIII defect, an X linked recessive defect (although factor VIII level normal twice) and exon 24 (C.3433G > A (p.Val1145Met) heterozygous with disease association of Gray platelet syndrome and autosomal recessive in nature. If the thrombocytopenia is due to a genetic condition that may be aggravated by ITP, then the platelet should not improve to the normal range (3.5 lac and above after steroid therapy). The acquired nature of the disease was again confirmed after falling in platelet up to 4000 with clinical bleeding after stopping steroid therapy.

Conclusions: This case highlighted the darker side of genetic testing that if the genetic report of the index case was available to us before the immunosuppressive therapy, we might not give the immunosuppressive therapy challenge to the index case, and he continued to remain thrombocytopenia and even may succumb to illness. Hence cautious use of genetic testing and appropriate clinical correlation is very important to use judiciously and optimally. Not to say that common causes are always common and always need to keep in mind while evaluating any clinical case and take timely clinical judgment for the benefit of the patient. Never carried away by genetic test reports by ignoring clinical clues, it can be very dangerous.

Evaluation of Platelet Indices in Patients of Thrombocytopenia and its Correlation with Bleeding Tendency

Karishma Makwane, R. K. Nigam

Introduction: The study aimed at investigating the role of platelet volume indices in the differential diagnosis of thrombocytopenia that may help in avoiding or delaying patients from undergoing unnecessary, invasive bone marrow aspiration or prevent undesirable platelet transfusion.

Aims & Objectives: Primary Objective:-Evaluation of platelet indices in patients of thrombocytopenia.

Secondary Objective:-To assess the sensitivity and specificity of these indices and set cut off values that will aid as predictor of bleeding in thrombocytopenic cases.

Materials & Methods: The study was conducted on a total 310 patients having thrombocytopinea. Blood sample was collected and subjected to estimation of platelet indices.

Result: Among cases with hypoproductive thrombocytopenia, mean platelet count were lowest in myelofibrosis (25,000 lakhs/cumm), Mean MPV count were lowest in patients with AML (8.17 ± 1.19 fl), mean PDW levels were lowest among patients with chronic lymphocytic leukemia (10.8 ± 0.8 fl), mean PCT levels and P-LCR levels were lowest in Multiple Myeloma (20.3 ± 4.2% and 0.01 ± 0.01% respectively). In hyperdestructive thrombocytopenia, mean platelet count were highest in malaria (52,714.2 ± 29,282.2 lakhs/cumm), mean P-LCR were highest in sepsis (0.09 ± 0.17%). Mean MPV, mean PDW, and mean PCT count were highest in immune thrombocytopenia i.e. 13.3 ± 1.3 fl, 17.2 ± 0 fl, and 51.1 ± 6.8% respectively. Area under the curve for platelet count and other indices was in lower range and are not considered as good tool for predicting the bleeding in our study.

Conclusions: Platelet indices can be used to discriminate the cause of thrombocytopenia as hypo productive and hyper destructive. These indices were found to have low sensitivity and specificity for becoming a predictive tool for thrombocytopenia. Thus, division of thrombocytopenia patients in hypoproductive and hyperdestructive, categories may help in the initial management of the conditions avoiding unnecessary interventions.

Comparative Study of Low Dose Vs High Dose Prophylaxis In Hemophilia: An Experience from aTertiary Centre in Eastern India

Moupali Ghosh, Maitreyee Bhattacharyya

Introduction: High dose factor prophylaxis in the dosage forms of > 25units/kg of body weight is an established standard of care for hemophilia in developed countries. However, in many studies from developing countries, low dose prophylaxis was found to be effective. There is limited data on comparison of low dose vs high dose prophylaxis from the developing countries.

Aims & Objectives: The purpose of the study was to compare low dose vs high dose factor prophylaxis in Hemophilia patients. Here we try to develop a protocol of low dose prophylaxis in developing countries.

Materials & Methods: The study was conducted at Institute of Haematology and Transfusion Medicine, Kolkata from April 2021 to August 2022. The study population was divided into two cohort, low and high dose prophylaxis patients. Low and high dose prophylaxis patients are those receiving < 25 IU/kg and > 25 IU/Kg factor infusion twice weekly. Clinical outcome between the groups was compared taking annual bleeding rate and joint health score into account.

Result: A total 109 patients aged 1 to 38 years receiving Hemophilia prophylaxis were included in the study. Eighty four percentage of the total population were severe hemophiliacs whereas 16% were moderate. Forty four percentage received low dose prophylaxis. Fifty seven percentage of the total population received primary prophylaxis whereas 43% received secondary prophylaxis. Annual bleeding rates of > 2 bleeds per year were found in 61.22% in low dose prophylaxis arm in comparison to 51.77% in high dose prophylaxis arm. Comparison of HJHS scoring revealed score of > 4 in 48.9% and 58.33% in the low dose and high dose prophylaxis arm respectively. Inhibitor screening was positive in 6% of hemophiliacs receiving low dose versus 11% of those receiving high dose prophylaxis.

Conclusions: A more intensive and high dose prophylactic regimen was found to be more effective; however, incidence of inhibitor positive was increased, as well as at a higher cost of treatment. Hence, low dose prophylaxis might be considered as a cost effective treatment in developing countries.

Cerebral Venous Thrombosis in a Patient with Immune Thrombocytopenia: A Clinical Paradox

Prerna Pramanik, Maitreyee Bhattacharyya

Introduction: Immune thrombocytopenia is characterized by immune mediated destruction and impaired production of platelets predisposing to bleeding mostly. However few cases of ITP has been seen to present with thrombosis.Multiple factors predispose patients to thrombosis in ITP. Patients with active disease are particularly at risk for paradoxical thrombosis due to increased turnover of platelets in bone marrow and higher levels of circulating platelets microparticles which promote thrombin formation and promote venous thrombosis.

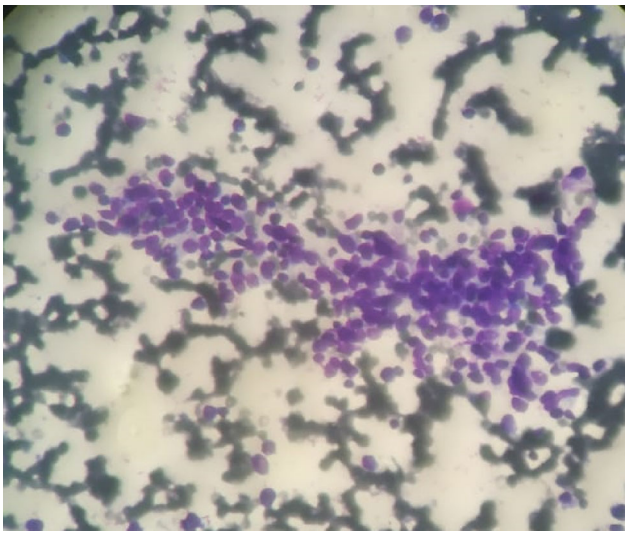
There are only few reported cases of cerebral sinus venous thrombosis (CSVT) in ITP.
Aims & Objectives: To evaluate the clinical profile and outcome of a newly diagnosed ITP patient presenting with CSVT.
Materials & Methods: It is a retrospective descriptive case report. The case report describes the clinical profile and outcome of a newly diagnosed ITP patient who had presented with headache in the ITP Clinic of IHTM.
Result: We present a paradoxical case of ITP who presented with cerebral venous sinus thrombosis. A 20 year old male, recently diagnosed ITP patient was treated initially with steroids and IVIG and was on regular follow up. He presented one month later with occipital headache and thrombocytopenia. Imaging showed cerebral venous thrombosis. He was started on methyl predisolone and simultaneously started on anticoagulation after 3 days. The patient improved both clinically and radiologically.
Conclusions: Any patient with clinical suspicion of thrombosis should be searched for in ITP. In patients with ITP and thrombotic events, judicious use of anticoagulation therapy is indicated along with simultaneous therapy directed at improving platelet count.

Bone Marrow Failure and MDS (Clinical)

A Case of Refractory Thrombocytopenia with Sinonasal Mass in SLE: A Diagnostic Dilemna

Prerna Pramanik, Maitreyee Bhattacharyaa

Introduction: SLE is a systemic autoimmune disorder that is frequently complicated by haematological manifestations such as hemolyticanemia, leukopenia and thrombocytopenia. Thrombocytopenia has been reported in 20% to 40% of patients with SLE and is usually attributed to an autoimmune mechanism similar to that of idiopathic immune thrombocytopenia (ITP). Here we present a case of severe thrombocytopenia which was not responding to any of the conventional therapies.
Aims & Objectives: To evaluate the clinical profile and outcome of an SLE patient presenting with a sinonasal mass and severe thrombocytopenia.
Materials & Methods: It is a descriptive case report. We report a case of 30 year old diagnosed case of female SLE patient on treatment and adequately controlled for two years. She initially presented with nasal stuffiness 4 months back, then developed a polypoidal mass arising from the nasal cavity, which extended up to the left maxillary antrum. She developed a significant episode of epistaxis. During this time she was found to have developed thrombocytopenia which was not responding to IVIG, steroids or platelet transfusion. She underwent biopsy from the sinonasal mass which was non contributory. She was then planned for bone marrow examination.
Result: Morphologically the bone marrow biopsy was initially reported as acute leukemia however immunophenotyping showed only 3% myeloid blasts. Then the bone marrow biopsy was reviewed which revealed the involvement by a metastatic deposit of some solid organ tumour and was confirmed by immunohistochemistry which was suggestive of Embryonal Rhabdomyosarcoma. She was transferred to Oncology Department for further management.
Conclusions: SLE is associated with an overall increased risk of malignancy including both hematological and solid organ cancers. However, cases of solid organ metastasizing to bone marrow and masquerading as isolated thrombocytopenia is exremely rare. Grade degree of suspicion is needed for its prompt diagnosis and management.



Clinical Profile and Treatment Outcome of Severe Aplastic Anemia in Adults: An Experience from Tertiary Care Centre in North India

Deepika Gupta, Priynaka Moule, Chetan Agarwal, YogalaxmiSivaprakasam, Ramesh Balasubramanian, Megha Verma, Jyoti Kotwal, Nitin Gupta

Introduction: Untreated/refractory severe aplastic anemia (SAA) is associated with very high mortality. Allogenic bone marrow transplantation or immunosuppressive therapy remains mainstay of treatment but these treatments are timely available to only a select subset of patients. Recently eltrombopag has been approved for treatment of SAA.
Aims & Objectives: We aimed to describe clinical profile and treatment response in patients with SAA from a tertiary care centre.
Materials & Methods: A retrospective analysis of patients diagnosed with SAA over a period of 7 years from January 2015-December 2021 was performed. The details of demographic profile, laboratory features, treatment given and response were analyzed.
Result: Ninety patients were diagnosed with SAA during this period out of which 18 patients went elsewhere for treatment. Seventy-two patients who received treatment in our hospital were included in the analysis. Sixty-two patients were SAA while 10 VSAA. PNH screening was done in 24 patients, out of which 17 (70%) had small clone. The details of treatment and response achieved is shown in Table 1. Eight patients (11.1%) received matched related donor allogenic hemopoietic cell transplant, out of which one had rejection followed by auto recovery while one died 6 months later due to covid 19 disease. Sixty-four patients received immunosuppressive therapy, forty-nine (76%) responded. Recurrence of SAA occurred in two patients who has achieved complete response to ATG therapy; one received second course of horse ATG + CSA + ETP and responded again.
Conclusions: Timely diagnosis and appropriate treatment selection is of utmost importance to achieve optimal outcome in severe aplastic anemia. Eltrombopag has become an important addition not only in front line but also in relapsed refractory aplastic anemia. Patients lacking donor, or resources for ATG should be treated with cyclosporine and eltrombopag as early as possible.

Table 1 Treatment received and response

Groups	N (%) Total N = 72	M:F	ATG + CSA N = 21	ATG + CSA + ETP N = 5	CSA N = 26	CSA + ETP N = 12	ALLO BMT N = 8
SAA	**62(86%)**	33:29	N = 18	N = 5	N = 24	N = 10	CR = 5
			CR = 7	CR = 1	CR = 10	CR = 4	GRAFT REJECTION = 1
			PR = 4	PR = 2	PR = 8	PR = 3	
			NR = 7	NR = 2	NR = 6	NR = 3	
VSAA	**10(14%)**	8:2	N = 3	0	N = 2	N = 2	N = 3
			CR = 1		PR = 2	CR = 1	CR = 3
			NR = 2			PR = 1	

CSA, cyclosporine A; ETP, eltrombopag; ATG, anti thymocyte globulin; BMT, bone marrow transplant

Pancytopenia with Hepatosplenomegaly: A Rare Case of Jacobson Syndrome

Sushma Yendamuri, Uday Yanamandra, Nikhil Tiwari, Sanjeev Khera, Deepti Mutreja, Preeti Tripati

Introduction: Bone marrow failure syndromes in infants is frequently a part of congenital syndromes.
Aims & Objectives: To present congenital causes of bone marrow failure syndromes.
Materials & Methods: The child was born as full-term baby by LSCS with uneventful antenatal and perinatal period presented with respiratory discomfort, increased sweating, failure to thrive, cyanotic spells, suck-rest-suck cycle since birth. Clinically he had loud P2 with ejection systolic murmur grade 3/6 with no parasternal heave and precordial bulge, hepatosplenomegaly 3 cm each below the costal margin with no signs of free fluid. On evaluation revealed pancytopenia with low platelet count (40,000 to 64,000) with normocytic normochromic anemia (Hb- 9gm/dl) and low absolute neutrophils (800/3000). He underwent 2 D ECHO which revealed large VSD, L-R shunt, severe PAH and LVVO. A bone marrow aspiration and biopsy was done to rule out storage and infiltrative disorders. Hemoglobin electrophoresis was done was normal. Bone marrow aspiration report revealed dysplastic changes in all cell lineages, myeloid precursors are markedly reduced in number with > 10lls in the form of hypolobation, pseudo pelgerhuet cells, hypogranularity and occasional ring forms, megakaryocytes are adequate and > 30% are dysplastic in the form of micro megakaryocytic, hypolobation and pawn ball appearance with increased histiocytes interstially with high suspicion for storage disorders hence beta glucosidase enzyme assay was done which was normal (2.74 nmol/hr/ml) and ruled out Gaucher's disease. A repeat bone marrow at 10 months of age was suggestive of refractory cytopenias of childhood. TORCH assay, CMV DNA PCR and ANA were negative. owing to a prolonged aPTT patient was evaluated for factor deficiencies which revealed markedly reduced factor XI functional assay (3.4%) with normal functional levels of Factor XII (127.4%). In view of above features of failure to thrive, developmental delay, pancytopenia with hepatosplenomegaly, cyanotic spells, and 2 DECHO showing large VSD, L-R shunt, severe PAH and LVVO suggesting congenital heart disease he underwent chromosomal analysis which showed microdeletion of chromosome 11q 24–25 deletion which was suggestive of JACOBSON'S SYNDROME.
Result: it is important to perform detailed multisystem evaluation through syndromic approach for all infants presenting with pancytopenia or bone marrow failure.
Conclusions: We present an interesting case of JACOBSONS SYNDROME presenting at 3 months of age with pancytopenia resulting in failure to thrive.

Hemophagocytic Lymphohistiocytosis-Macrophage Activation Syndrome (HLA-MAS) as the First Presentation of Systemic Lupus Erythematosus: A Rare Case Report

Pankaj Sukhadiya, Durga Shankar Meena, Mahendra Kumar Garg, Pawan Garg, Akhilesh Kumar PH, Neeraja Vijayan, Pranav Kumar

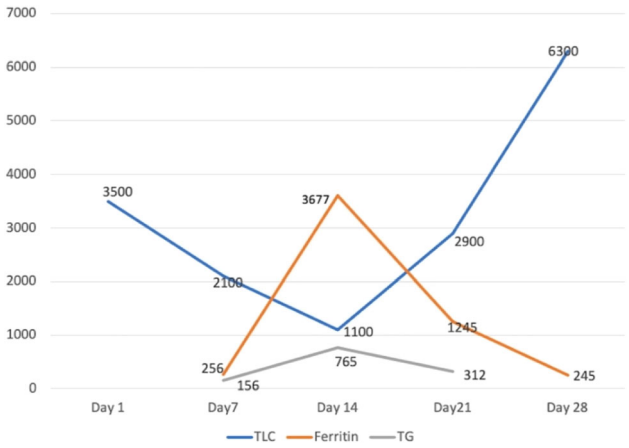
Introduction: HLH is a clinical syndrome characterised by abnormal and excessive immune activation and tissue inflammation. Lack of normal downregulation of tissue macrophages results in cytokine storm and tissue damage. HLH can present as an isolated event or multiple episodes, as seen in familial HLH. Any alteration in immune homeostasis could trigger HLH. SLE is now emerging as an important aetiology in HLH cases, with a reported prevalence of 0.9% to 10%. We highlight a case of a young female who was admitted with pyrexia of unknown origin (PUO) and, later on during hospitalisation, developed HLH and subsequently SLE.
Aims & Objectives: We highlight possibility of HLH as an initial presentation of underlying SLE and need of early diagnosis and management of HLH-MAS, which can be sole presentation of autoimmune diseases.
Materials & Methods: We are presenting a case of 25 years old female, admitted in General Medicine ward at AIIMS JODHPUR. Clinical data, laboratory data and radiographic details were evaluated. Diagnosis was made according to HLH 2004 Trial criteria. Patient was managed and further monitored according to HLH 2004 trial.
Result: This patient presented with febrile illness for two weeks with subsequent cytopenias. Initially, she was evaluated in the line of PUO (pyrexia of unknown origin). Later on, she developed psychosis, seizures and respiratory distress. The differential diagnosis was broad, including infections, malignancy, or autoimmune diseases. Infectious aetiology was very high in the differential diagnosis, and a complete workup was done, although negative. Notwithstanding, a bone marrow biopsy revealed *Candida parapsilosis*; however, her blood cultures were repeatedly negative, and we did not find any other evidence of disseminated candidiasis. The patient was diagnosed with HLH fulfilling five out of eight clinical/laboratory diagnostic criteria, including fever, pancytopenia, hypofibrinogenemia, splenomegaly and hyperferritinemia. Following which patient was also diagnosed with SLE according to ACR-EULAR 2019 criteria, making it HLH-

MAS. We used the HLH- 2004 protocol for the management of this patient. After 2 weeks of therapy, patient's clinical and laboratory profile got improved.
Conclusions: HLH sometimes be the first presentation of underlying autoimmune disorders, as highlighted by this report. Early suspicion and use of aggressive immunosuppressants is the key to improving survival. Moreover, the therapeutic regimen should be individualized based on clinical response, underlying trigger, comorbidities and possibilities of adverse events.

Bone Marrow Failure and Combined Immunodeficiency: A Rare Case of DNA-Ligase 4 Deficiency

Nitin Chaudhary, Gopinathan M, Priyanka Chauhan, Faheema Hasan, Sanjeev, Anshul Gupta, Rajesh Kashyap

Introduction: DNA Ligase IV (LIG4) syndrome is a rare disease in which patients can present with microcephaly, growth retardation, developmental delay, dysmorphic facial features, combined immunodeficiency, hypoplastic bone marrow and predisposition to malignancy. LIG4 syndrome is caused by homozygous or compound heterozygous mutations in the LIG4 gene. Although this disease was first described nearly 30 years ago, only a few cases have been reported to date.
Aims & Objectives: We hereby report the course of a 4-year-old female child with distinctive clinical features of LIG4 Syndrome.
Materials & Methods: The child was born to non-consanguineous parents as term, short for gestational age with otherwise normal natal history and normal developmental milestones. She developed fever 4 months back and found to have pancytopenia on CBC requiring PRBC transfusion support. She also had recurrent upper respiratory tract infections requiring IV antibiotics. She also had repeated episodes of diarrhea with no definite foci of infection presumably immunological in nature. Child was noted to have failure to thrive, short stature, microcephaly with bird like facies. She also had a large capillary hemangioma behind her left ear. She was evaluated for pancytopenia and found to have severe aplastic anemia on bone marrow examination. PNH Clone was negative. Stress Cytogenetics came out to be negative. NGS was sent in view of inherited bone marrow failure syndrome which showed deletion of exon 3 of LIG4 gene (c.597-600del).
Result: The child was counselled for upfront transplant and while evaluating the younger brother as a prospective donor, he was found to have similar phenotypic features. Genetic testing of the brother also revealed the same mutation. Family screening and genetic counselling was advised for future progeny. LIG4 Syndrome is characterized by failure to thrive, growth retardation, microcephaly, narrow forehead, hypotelorism, prominent nose cryptorchidism, amenorrhea, photosensitivity, global developmental delay, delayed speech, hypothyroidism, pancytopenia, thrombocytopenia and myelodysplasia. She was started on anabolic steroids with transfusion support till allogenic stem cell transplant.
Conclusions: Patient presenting with microcephaly, growth retardation, immunodeficiency and pancytopenia should be evaluated for inherited bone marrow failure syndromes like LIG4 Syndrome.



Fanconi Anemia with Acute Myeloid Leukemia: An Uncommon Cause of Pancytopenia in a Child

Jayita Das, Prita Pradhan, Suravi Mohanty, Urmila Senapati, Palash Das, Sulagna Sahoo, Ranjita Panigrahi

Introduction: Fanconi anemia (FA), an uncommon autosomal recessive disease, manifests a myriad of congenital anomalies and diverse hematological disorders.
Aims & Objectives: Here we present a case of Fanconi anemia and acute myeloid leukemia presenting with pancytopenia.
Materials & Methods: A 9-year-old female child presented with petechial rash and weakness since 3 months. Physical examination revealed bilateral thumb hypoplasia, Café au lait macules and absent radial pulse. Chromosomal breakage in mitomycin culture reported 100% breaks, triradials and quadrilaterals.
Result: Complete blood count revealed pancytopenia with 8% atypical cells. Bone marrow revealed suppression of marrow with increased number of blasts. Blast cells are positive for myeloperoxidase. Flow cytometry showed MPO, Tdt, CD13 and CD33. Diagnosis of acute myeloid leukemia with monocytic differentiation was confirmed. Child was referred to higher Centre for further treatment.
Conclusions: FA is a rare bone marrow failure syndrome, which may also present with leukemia as highlighted in the present case. Careful monitoring of blood counts, breakage analysis and flow cytometry in conjunction with detailed clinicoradiological evaluation can help early diagnosis and management.

ATG In Aplastic Anemia: Our Experience

Santosh Kumar, Avinash Kr Ssingh, Santosh Kumar, Jitendra Kumar, Khursheed Mallick, Ankit Kumar, Divya Krishna

Introduction: Aquired aplastic anemia is characterised by pancytopenia with hypocellular bone marrow in the absence of fibrosis or infiltration.Sibling matched stem cell transplant is best modality of treatment for young patients (less than 40 yrs) and ATG with cyclosporine with or without revolade is recommended for older patients or patients without sibling matched donor.
Aims & Objectives: we wished to share our experience of ATG in acquired aplastic anemia patients.
Materials & Methods: Data collected from hospital records, all patients received 40 mg/kg/day for 4 days of ATGAM (PFIZER) with cyclosporine 5 mg/kg/day in 2 divided doses since day 14

onward. Received 2 weeks of corticosteroid to prevent serum sickness secondary to ATG.Response criteria were as per standard guidelines. Last 5 pt thymogam.

Result: Total 15 patients received ATG PLUS CYCLOSPORINE IN LAST 3 years. Male:female ratio was 9:6. Median age was 52 yrs. All patients tolerated ATG well except for mild chills, rigor and fever which responded to antihistaminic, hydrocortisone and paracetamol and temporary discontinuation of ATG. Out of 15 patients, 11 has received 6 months of Elrombopag along with ATG plus cyclosporine. 6 patients (60%) achieved complete response each after 6 month and 1 year respectively. 4 has achieved partial response after 6 month, 5 patient has no response 3 years after ATG. Follow up duration ranged from 10 months to 6 years. We lost 2 pts due to neutropenic sepsis patient with minimal response expired due to neutropenic sepsis among non-responders.Overall response is 60% (CR + PR).

Conclusions: ATG + CYCLOPSPORINE WITH OR WITHOUT ELTROMBOPAG IS A better treatment option and life saving for our patients who doesn't have matched sibling or are not fit for stem cell transplant. With the huge number of aplastic anemia in our state and poor response with cyclosporine alone, early start of ATG plus cyclosporine can benefit.

Anti-Thymocyte Globulin-Based Immunosuppressive Therapy in Acquired Aplastic Anemia: Long Term Outcomes

Sohini Chattopadhyay, Sharon Lionel, Sushil Selvarajan, Anup J Devasia, Anu Korula, Uday Kulkarni, Fouzia NA, Kavitha M Lakshmi, Alok Srivastava, Aby Abraham, Vikram Mathews,Biju George

Introduction: Acquired Aplastic anemia (AA) characterized by the failure of the hematopoietic system often leads to fatal outcomes without appropriate therapy. Immunosuppressive Therapy (IST) remains the corner stone of treatment in acquired aplastic anemia in patients without a matched related donor.

Aims & Objectives: We describe our experience with antithymocyte globulin therapy (ATG) in combination with Cyclosporine (CsA) in patients with AA.

Materials & Methods: This is a retrospective analysis done at CMC Vellore, describing the outcomes of adult patients with AA treated with IST. Patients were classified into Non-severe, Severe, and Very Severe AA based on standard IAASG criteria.

Result: Between 1989 and 2020, a total of 628 patients diagnosed with AA, received ATG ± CsA. Of these, data was unavailable in 141 patients, 71 patients were lost to follow-up, and 53 patients had died within a year of receiving IST and were excluded from the study. 363 patients were included in the study. Baseline characteristics are summarised in Table 1. At 3 months, the overall response rate (ORR) was 57%, with 2.5% CR (complete response) and 54% PR (Partial Response). At 6 months, the ORR was 74%, with 13% CR and 61% PR. Amongst the 96 (26%) non-responders, 15 (4%) attained response with continuation of CsA, 38 (10.5%) attained response with addition of androgens, 6 (2%) responded to a combination of androgen + thrombopoietin agonists. Eleven patients among the remaining 37 patients, attained response with a stem cell transplant. Adverse events in the form of hypertension(33%), serum sickness (23%), acute kidney injury requiring discontinuation of CsA (13%), Avascular necrosis (11%), gingival hyperplasia (4%), and diabetes mellitus (5%) were encountered.

On follow-up, a relapse of AA was noted in 89 patients (24.5%). Clonal evolution in the form of paroxysmal nocturnal hemoglobinuria was seen in 25 patients (6.8%), 8 patients (2.2%) developed myelodysplastic syndrome and 8 patients (2.2%) progressed to acute myeloid leukemia.

Conclusions: Immunosuppressive therapy is an effective treatment option in patients with AA providing an excellent hematological response. However, the duration of immunosuppression requires optimization to sustain response in patients.

Table 1: Baseline Characteristics

Patient Characteristic (N=363)	**N(%)/ Median (Range)**
Age at Diagnosis	39 years (16-74 years)
Gender (Male/Female)	235 (65%)/ 128 (35%)
Aplastic Anemia Severity -Non severe -Severe -Very Severe	 102 (28%) 202 (56%) 59 (16%)
No of Transfusions	13 (0-90)
Prior Treatment -Cyclosporine (CsA) -Androgen based therapy (Stanozolol/Danazol/Oxymetholone) -CsA+ Androgen -Androgen + Thrombopoietin (TPO) Agonist	 106 (29%) 65 (18%) 51 (14%) 10 (2.8%)
Median time to Treatment with ATG from Diagnosis	6 months (1-204 months)
ATG Treatment Details -ATG+CsA -ATG+ CsA+ TPO Agonist (Romiplostim/ eltrombopag) -ATG	 331 (91%) 9 (2.5%) 23 (6.5%)

Efficacy of Triple Drugs Combination of Hatg, Cyclosporine and Eltrombopag in Primary Severe Aplastic Anemia (PSAA): Experience From Prospective Study in Single Institution

Sandeep Kumar Prusty, M. Biswal, J.K Panda, R.K. Jena

Introduction: Aplastic anemia is a common disease in South Asia. Allogenic sibling BMT is the treatment of choice for patients < 50 years and hATG and cyclosporine for > 50 years. Attempts has been made to improve the results by addition of new drug, Eltrombopag to the double drug regimen.

Aims & Objectives: To study the efficacy of triple drugs combination of hATG, Cyclosporine and Eltrombopag in PSAA.

Materials & Methods: A prospective study in the Clinical hematology Dept. SCB MCH involving 96 cases of PSAA. All drugs and supportive therapy are supplied by Govt. of Odisha at free of cost.

Drugs- hATG, Eltrombopag and Cyclosporine.

Supportive therapy: PRBC and platelet transfusion and other therapy as and when required.

Inclusion Criteria:

1. All diagnosed cases fulfilling the criteria of PSAA of > 50 years of age and cases < 50 >

2. Patient willing to give informed consent.

Exclusion Criteria:

1. All cytopenia cases other than PSAA.

2. Cases having cardiac, liver and renal functions impairment.

Primary end point: Type of response at the end of 6 months.

(a) CR: when- Hb > 10 g%, ANC > 1000, TPC > 100,000.

(b) NR: Absence of all 3 above criteria's.

(c) PR: Parameters in between CR and NR.

(d) ORR: CR + PR.

Evaluation:

1. CBC—weekly for 1st month and then bi-weekly for 12 months.

2. LFT, RFT, FBS, Viral markers and electrolytes—monthly.

End of study:

All the patients followed for 12 months and evaluated and categorized according to the response as CR, PR, NR and ORR.

Result: Largest prospective study in India utilizing triple combination of hATG (Indian Make), cyclosporine and eltrombopag.

The response rate was reported at 6 months and 12 months were respectively CR 40.62%, PR 38.54%, NR 6.25%, death 14.58% and ORR 79%, and CR 45.83%,PR 35.41%, NR2.08%, death 16.66% and ORR 81.25%.

The optimum response was reported at the end of 1 year.

Drug toxicity and interruption were in the minority of cases and manageable.

Conclusions: The triple drug therapy of hATG, cyclosporine and eltrombopag showed better result of hematopoeisis than the double drug combination therapy hATG and cyclosporine. It implies that Eltrombopag exerts a synergistic effect and enhance thrombopoeisis, erythropoeisis and granulopoeisis.

Criteria	No (%) @ 3 m	No(%) @ 6 m	No (%) @ 12 m
CR	11 (11.45)	39 (40.62)	44 (45.83)
PR	23 (23.95)	37 (38.54)	34 (35.41)
NR	48 (0.5)	6 (6.25)	2 (2.08)
ORR	34 (35.41)	76 (79.16)	78 (81.25)
Death	14 (14.58)	14 (14.58)	16 (16.66)

Outcome of Immunosuppressive Therapy in Patients with Acquired Aplastic Anaemia: Experience from a Tertiary Care Hospital in West Bengal

Chirasree Sanyal, Kaustav Ghosh, Shipla Roy, Abhishek Maurya, Apurva Baneerjee, Subham Bhattacharya, Shuvra N. Baul, Sandeep Saha, Prakash K Mondal, Rajib De, Tuphan K. Dolai

Introduction: Aplastic anaemia (AA) is an immune-mediated bone marrow failure disorder and most AA patients unable SCT due to co-morbidity and financial reasons.

Aims & Objectives: The aim of the study was to assess response of horse ATG (ATGAM & THYMOGAM) and cyclosporine.

Materials & Methods: This prospective study was conducted from year March 2011 to August 2022.AA was diagnosed as per established criteria. ATG was administered at the dose of 40 mg/kg/day for 4 days and cyclosporine at 5 mg/kg/day at two divided doses from day 14 onwards. Response assessment was done as per published criteria.

Result: Among 107 patients, median age of patients was 37 years (8–66 yrs). Non severe AA were 17 (16%), severe AA 84(78%) and very severe AA 6 (6%). Median duration from diagnosis to ATG therapy was378 days (10–1825)71.1% patients received THYMOGAM and28.9% received ATGAM.

Overall response at 3 months after initiation of IST was 42.9% (46/107), partial remission PR (42.2%), complete remission CR(2.9%)and overall response at 6 month was 64.1% (59/92), PR (52.1%), CR(7.3%).Overall survival at 6 months follow-up after IST was 89.7%.

3 months response for ATGAM PR (48.5%), CR (6.1%) and for THYMOGAM PR(39.1%),CR(1.4%).

6 months response for ATGAM PR (45.6%), CR (18.2%) and for THYMOGAM PR(55.6%), CR(1.6%).

Overall response rate(ORR) after 6 months is 63.8% with ATGAM and 57.2%with THYMOGAM.

Conclusions: Our study shows an overall survival of 89.7% at a median follow-up of 6 months and the variables that significantly affected overall survival were time from diagnosis to ATG therapy, response to therapy at 3 months ($p = 0.238$) and at 6 months ($p = 0.012$) and advance age group, presence of co morbidities and occurrence of complications.

A Wave Of HLH!: Outcomes in a Cluster Of Malignancy-Associated Secondary Hemphagocytic Lymphohistiocytosis

Shruti Bhise, Hemant Menghani, Manthankathrotiya, Sandipkheni, Jayanipatel, Ankitjitani, Vijayshirure, Velu Nair

Introduction: Hemophagocytic Lymphohistiocytosis (HLH) is an aberrant hyperinflammatory immune response syndrome that can lead to a potentially fatal cytokine storm. Malignancy-associated HLH (Mal-HLH) is rare and can complicate the preexisting illness, leading to fatal outcomes. We present 3 cases with Mal-HLH, their diverse clinical presentation and outcomes following treatment with HLH 2004 protocol.

Aims & Objectives: To observe the response, outcomes and complications of Mal-HLH following treatment with HLH 2004 protocol while simultaneously treating the underlying cause.

Materials & Methods: In April 2022, three cases presented with persistent fever and pancytopenia. Case 1 had Myelodysplastic syndrome Excess Blasts -1, RIPSS -high risk on Azacitidine and Venetoclax therapy with disease remission and presence of EBV viremia. Case 2 had Acute myeloid leukemia FLT3-TKD, NPM1 mutated,46XX, ELN- intermediate risk post-induction chemotherapy in morphological remission but with Minimal residual disease (MRD) positive. Case 3 had of T cell histiocyte-rich large B cell lymphoma stage 4, IPI- high risk with disease relapse post autologous stem cell transplant (AutoHCT) and on salvage chemotherapy. Extensive infective workup was negative, and fever was unresponsive to antimicrobials. HLH was suspected and patients were evaluated as per 1994 HLH diagnostic criteria followed by primary disease status evaluation. Treatment and assessment of the outcomes was done on the lines of the HLH 2004 protocol.

Result: Patient characteristics and parameters with outcomes are shown in table 1. At 2 weeks, case 1 and case 2 showed a partial response short of a clinical response while case 3 showed a complete clinical response. At 8 weeks, case 1 had flare of HLH activity and case 2 endured a RhinocerebroorbitalMucormycosis, both succumbed to complications of the above, while case 3 had complete resolution of HLH. At 11 weeks, case 3 contracted a COVID 19 infection followed by HLH reactivation at 14 weeks and is currently under treatment for the same.

Conclusions: Mal-HLH can have varied precipitating causes other than the underlying disease itself leading to fatal outcomes despite adequate treatment. Hence, a high index of clinical suspicion, timely evaluation and aggressive treatment of the underlying cause is prudent.

	Case 1	Case 2	Case 3
Age(yrs)	49	53	28
Sex	Female	Female	Male
Primary diagnosis	MDS EB1 RIPSS-High risk	AML, FLT3 TKD, NPM1, intermediate risk	TCHRBCL, stage 4, IPI- high risk
Treatment	On Azacitidine 7th cycle. Planned for HCT	Post induction chemotherapy On Azacitidine 1st cycle Planned for HCT.	Post Auto HCT on salvage chemotherapy (RGDP)
Disease status	Remission	Morphological Remission (MRD positive)	Relapse
EBV PCR	Positive (85 copies)	Negative	Negative
AT DIAGNOSIS			
Clinical criteria (2004 HLH criteria)			
Fever	+	+	+
Splenomegaly	+	-	+
Laboratory criteria (2004 HLH criteria)			
Cytopenia	+	+	+
Ferritin(ng/L)	6803	5586	5992
Fibrinogen(mg/dL)	288	141	153
Triglyceride(mg/dL)	262		348
Il2 R(U/ml)	3496	3496	1720
Bone marrow examination	-	Hemophagocytosis	-
RESPONSE AT 2 WEEKS			
Clinical criteria (2004 HLH criteria)			
Fever	-	-	-
Splenomegaly	+	-	+
Laboratory criteria (2004 HLH criteria)			
Cytopenia	+	+	-
Ferritin (ng/L)	2619	1975	1287
Fibrinogen (mg/dL)	191	324	-
Triglyceride(mg/dL)	187	374	275
Il2 R (U/ml)	2432	1294	-
	Partial response	Partial response	Complete Clinical response
RESPONSE AT 8 WEEKS			
Clinical criteria (2004 HLH criteria)			
Fever	+	+	-
Splenomegaly	+	-	-
Laboratory criteria (2004 HLH criteria)			
Cytopenia	+	+	-
Ferritin (ng/L)	15515	3133	487
Fibrinogen (mg/dL)	273	324	-
Triglyceride(mg/dL)	187	137	275
Il2 R (U/ml)	6217	1298	-
	Death due to complications related to disease (HLH) flare.	Death due to rhino-orbito-cerebral mucormycosis secondary to persistent cytopenia	Alive and resolution of HLH
OUTCOME AT 11 WEEKS			
			COVID 19 infection
OUTCOME AT 14 WEEKS			
Clinical criteria (2004 HLH criteria)			
Fever			+
Splenomegaly			+
Laboratory criteria (2004 HLH criteria)			
Cytopenia			+
Ferritin(ng/L)			16990
Fibrinogen(mg/dL)			80
Triglyceride(mg/dL)			144
Il2 R(U/ml)			-
			HLH reactivation

MDS-Myelodysplastic syndrome, EB- Excess blasts, IPSS- Revised international prognostic scoring system, AML-Acute Myeloid leukemia, FLT3 TKD- Fms related receptor Tyrosine kinase 1 Tyrosine kinase domain, NPM1- Nucleophosmin, European Net, TCHRBCL-T cell histiocyte rich large B cell lymphoma, International Prognostic Index, RGDP-Rituximab Gemcitabine Dexamethasone Cyclophos, MRD-Minimal residual disease, HLH-Hemophagocytic lymphohistiocytosis, IL2-Interleukin2 receptor

Linezolid-Induced Reversible Myelosuppression with Multilineage Dysplasia and Ring Sideroblasts

Shruti madan, Hari Neupane, Shelly Singla, Lekshmon Kalikkaleth Sasidharan Pillai, Sreejesh Sreedharanunni

Introduction: Several reversible causes, including drugs, can produce clinical and hematological findings.
mimicking myelodysplastic syndrome/MDS.
Result: A 23-year-old male, a case of C3 glomerulopathy on treatment for multidrug-resistant tuberculosis using Bedaquiline, levofloxacin, linezolid, clofazimine, and cycloserine for 1.5 months was evaluated for recent onset and progressive pancytopenia. His hemoglobin was 540 gm/L, total leucocyte count 3.2 × 10 6 /L, absolute neutrophil count 1.7 × 10 6 /L and platelet count-62 × 10 6 /L. Peripheral-smear examination showed microcytic hypochromic red cells. Bone-marrow was hypocellular with stromal degenerative changes, monolobated megakaryocytes (40%), hypolobated granulocytes with abnormal chromatin clumping (15%), vacuolated erythroid cells, ring sideroblasts/RS. (15%), iron overload (PERLS stain 5 +), occasional hemophagocytosis, and a healed granuloma. There were no cytogenetic abnormalities. In view of multilineage dysplasia and RS., a diagnosis of myelodysplastic syndrome was initially considered. However, since the patient was on linezolid therapy, reversible linezolid- induced myelosuppression and myelodysplasia were considered. His anemia (94 g/L) improved, TLC (6 × 10^9 /L), and PC (1.54 × 10^9 /L) became normal within four weeks of stopping linezolid with stable counts after one year of follow-up.
Conclusions: Erythroid vacuolations and RS are reported as clues for linezolid toxicity. Our patient had not only e throid vacuoles and RS., but also hypocellular bone-marrow withgranulocytic and megakaryocytic changes mimicking myelodysplastic syndrome. Our case highlights the importance of taking detailed clinical history and exclusion of reversible causes especially of drug intake, including indigenous medications, and exclude them as the possible culprit before considering invasive investigations and a diagnosis of MDS.

A Study on Outcome in Patients of Myelodysplastic Syndrome Treated with Inj Azacitidine

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Introduction: Myelodysplastic syndromes (MDS) are clonal haematopoietic stem cell disorders, characterized by dysplasia leading to cytopenias and a high probability of progression to acute myeloid leukaemia (AML). In 2004 the US Food and Drug Administration approved the hypomethylating drug Azacitidine for the treatment of MDS, mainly based on the significant delay in time to transformation to AML and death, compared with best supportive care. Azacitidine is a nucleoside analog that covalently binds to the DNA methyltransferases, irreversibly inhibiting their function, leading to the progressive loss of methylation and and reversal of gene silencing.
Aims & Objectives: To assess the outcome in patients of myelodysplastic syndrome treated with InjAzacitidine.
Materials & Methods: A Prospective interventional study done in patients of myelodysplastic syndrome attending the OPD, IPD and day care of department of Hematology, NRSMCH during the study period September 2021–August 2022. All age group patients were included after taking informed consent. Patients with major comorbidities and those who progressed to AML were excluded. Risk stratification was done as per Revised International Prognostic Scoring System for MDS. InjAzacitidine was given @75 mg/m2 for seven days subcutaneously every four weeks for six cycles and outcome was assessed in terms of overall survival, hematological improvement and frequency of transfusion.
Result: Ten patients of MDS with median age 53 years were included in the study. Eight patients were male and two female. Three patients each belonged to low and intermediate risk group and two patients were high risk group. Seven patients completed six cycles of InjAzacitidine, one patient died during the study period and two patients were excluded as they progressed to AML. Overall survival was 70% and hematological improvement along with transfusion independency was observed in 60% of the patients.
Conclusions: InjAzacitidine is effective in improving the overall survival, hematological improvement and frequency of transfusion in patients with myelodysplastic syndrome irrespective of risk group.

Bone Marrow Failure and MDS-Laboratory

Aplastic Anemia: Simple Yet Complicated: Our Experience

Sushma Belurkar, Harika Methuku

Introduction: Aplastic anemia (AA) is a rare hematological disorder defined by peripheral blood pancytopenia and hypocellular bone marrow. Though several studies have been done on pathophysiology, it is still not completely well characterized. AA is a challenging disease in a developing country like India. Main challenges faced are relatively longer time lapse between diagnosis and treatment and the economic constraints faced during the therapy.
Aims & Objectives: To study the prevalence of aplastic anemia in a tertiary care centre with clinico-hematological correlation.
To study response to treatment and prognosis in these patients.
Materials & Methods: This study includes 52 AA patients diagnosed on bone marrow morphology over a period of 5 years.Detailed clinical history including occupation history, medical history, drug history and physical examination findings were retrieved from the

patient files from the medical records department. Lab parameters were retrieved from the lab information system. Peripheral smear, Bone marrow aspirate and biopsy slides were analysed in each patient.
Result: Overall prevalence of AA was 0.7% with a median age of 48.5 years. M:F ratio was 1.73:1.The Median Hb value was 5.8 gm/dl,Mean corrected reticulocyte count was 0.34%,Mean absolute reticulocyte count (ARC) was 19.4 $\times$ 10^9/L,Median total leukocyte count was 2.4 $\times$ 10^3/μL,Median platelet count was 10 $\times$ 10^9/L,Mean absolute lymphocyte count (ALC) was 1.75 $\times$ 10^9/L and Absolute Neutrophil Count ranged between 0.5 and 1.5 $\times$ 10^9/L71.15.
Conclusions: Acquired causes of AA are more common than the congenital causes. High baseline ARC, ALC values and younger age of the patient are the factors which are associated with better response to treatment.Though allogeneic stem cell transplant is the definitive line of treatment, supportive treatment remains the mainstay treatment in developing countries like India.

An Evaluation for Clonal T Cells in Patients with Adult Onset Hypoproliferative Anemia with Erythroblastopenia and/or Bone Marrow Lymphoid Nodules

Tharageswari S, Sudhanshi Raina, Anand Balakrishnan, Pramod Singh, Parveen Bose, Sreejesh Sreedharanunni, Praveen Sharma, Pulkit Rastogi, Nabhajit Mallik, Narender Kumar, Prashant Sharma, Shano Naseem, Man Updesh Singh Sachdeva, Jasmina Ahluwalia, Reena Das, Deepesh

Introduction: The diagnosis and management of adult onset hypoproliferative anemia (anemia with corrected reticulocyte count $< 2\%$ or absolute reticulocyte count $< 10{,}000/\mu l$) is met with challenges. Clonal T cells are reported to cause immune mediated anemia (with or without erytrhoblastopenia) in a proportion of these cases.
Aims & Objectives: To study the frequency of clonal T cells occurring in adult onset hypoproliferative anemia with erythroblastopenia and/or bone marrow lymphoid nodules.
Materials & Methods: Over a period of one year (July 2021 to August 2022), a total of thirteen patients with hypoproliferative anemia (n = 13) having erythroblastopenia and/or bone marrow lymphoid nodules underwent T cell clonality testing by PCR using T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 kits (Invivoscribe Inc, USA) followed by fragment length analysis in ABI 3500 genetic analyzer. A 10-color flow cytometry for B and T cells (bone marrow = 11; peripheral blood = 1) was also performed in 12/13 cases.
Result: The age of patients ranged from 21–75 years (median:54 years). There were seven females. The hemoglobin (g/dl), total leukocyte count ($\times$ 106/L), absolute neutrophil count ($\times$ 106/L), and platelet count (109/L) ranged from 5.1–8.9 (median-6.9), 4.1–14.6 (median-6.1), 1.1–11.2 (median-3.2), and 0.33–4.69 (Median-1.82) respectively. The reticulocyte production index (%) and absolute reticulocyte count ($\times$ 1012/L) ranged from 0.01–1 (median-0.19) and 1.5–71.6 (median-7.7) respectively. One case had relative lymphocytosis (57%) while, none had large granular lymphocytosis ($> 2 \times 106$/L). Nine cases had erythroblastopenia in the bone marrow and seven cases had lymphoid nodules. Flow cytometry identified abnormal T cells in six cases (53.8%; CD8 + in four; gamma delta T cells in one and CD4-CD8- in one case) of which five cases showed monoclonal peaks in T-cell receptor gamma gene rearrangement assay, while one showed oligoclonality. The remaining cases showed polyclonal T cell population.
Conclusions: T cell clonality assay and flowcytometry detected monoclonal or oligoclonal T cells in 46.

High Frequency of MPL Gene Mutations in Young Aplastic Anemia

Sumithra Rajesh S, Arun KumarA, Eswari S, Uday Kulkarni, Fouzia NA, Aby Abraham, Vikram Mathews, Biju George, Eunice S Edison

Introduction: Aplastic anemia (AA) is characterised by hypocellular marrow resulting in pancytopenia. Recent studies have focused on identifying genetic variants causing bone marrow failure syndromes.
Aims & Objectives: In this study we attempted to identify germline variants in young aplastic anaemia patients.
Materials & Methods: AA patients below the age of 40 years who presented to the department of Haematology, CMC between 2018 and 2022 were included in the study. DNA was extracted and variants were screened either by Sanger sequencing or targeted next generation sequencing (NGS). Following library preparation with Illumina TruseqTM Nano DNA Library prep kit, sequencing was done on the Illumina platform. Bioinformatics was done following the GATK best practices framework. The variant screening was restricted to 434 genes associated with bone marrow failure and primary immunodeficiency. Bi-directional Sanger sequencing using target specific primers was performed in samples which were not processed for NGS. VNTR analysis was performed by fragment length analysis method.
Result: A total of sixty-nine patients were included in the study with a median age of 26 years (1–40). Genetic variants in fifty-four samples were analysed using next generation sequencing and direct sequencing of MPL gene was done in fifteen patients. MPL gene variants were found in 11/69 patients, with seven showing homozygous variants and three showing heterozygous variants. One case had a compound heterozygous variant. Variants were of missense type (5/11) followed by deletion (4/11) and splice-site (1/11). The case with compound heterozygous variants had a deletion and a splice-site variant. Ten of the eleven variants seen were pathogenic based on ACMG classification and one was a variant of Uncertain significance. Of these eleven patients, two were lost to follow up. Nine patients underwent HSCT of whom three survived and the rest succumbed.
Conclusions: MPL gene variants are a frequent cause of (15%) aplastic anemia in our cohort. Screening for germline genetic variants in young aplastic anemia patients may lead to better treatment strategies.

Clinicopathological Study of Aplastic Anemia and its Correlation with PNH Clones Detected by Flaer Based Multi Parametric Flow Cytometry

Prabhudatta Dash, Sarita Pradhan,. Priyanka Samal, Rajesh Kumar Bhola, Raka Hota, Ripunjaya Mohanty, Debahuti Mohapatra

Introduction: Bone marrow failure has been regarded as one of the triad of clinical manifestation of paroxysmal nocturnal hemoglobinuria (PNH) & PNH in turn has been descried as a late clonal disease evolving in patients recovering from Aplastic anemia(AA). The incidence of AA appears to be two to three fold more common in Asia than in Europe. Presence of minor PNH clones in AA is said to be associated with better response to immunosuppressive therapy. There is paucity of literature regarding the prevalence & clinicopathological correlation of PNH clones in AA in eastern India.

Aims & Objectives: 1. To enumerate the frequency and clone size of PNH in all cases of AA using FLAER based flow cytometry.
2. To compare the clinicopathological findings and disease severity between cases of Aplastic Anemia with PNH clones & AA without PNH clones.
Materials & Methods: This is a prospective study carried out in the Department of Hematology, IMS & SUM Hospital, Bhubaneswar from January 2020 to July 2022. Peripheral blood samples from cases diagnosed as AA on bone marrow biopsy, were tested for PNH clones using a panel of FLAER/CD 15/CD 64/CD 45/CD 24/CD 14 for granulocyte & monocytes & CD 235/CD 59 for RBCs respectively according to ICCS/ESCAA consensus guidelines. All relevant clinical and laboratory data were collected. Patient characteristics of aplastic anemia with PNH clones and without clones were compared.
Result: Out of 41 cases of AA, 19 (46.34%) cases showed presence of PNH clone which were subclinical (clone size $< 1\%$) in 21.05%, small clones (1–10%) in 26.31%, moderate clone (clone size 10–30%) in 31.57% & large clone (clone size $> 30\%$) in 21.05.
Conclusions: Presence of small PNH clones is not uncommon in Aplastic anemia and they differ in their clinicopathological attributes from AA without PNH clones.

Biological Pathways Associated with UNCLASSIFIED IBMFS

Phaneendra Datari, Alpesh Kapadia, Ashish Babu Gorantla, GauravJoshi, Arun Kumar Arunachalam,Uday Prakash Kulkarni,Fouzia N A, BijuGeorge, Eunice Sindhuvi Edison, Shaji R Velayudhan

Introduction: Inherited Bone Marrow Failure Syndromes are characterized by early onset of disease, physical abnormalities, overlapping clinical presentation and pathogenic variants in causative genes. With Next Generation Sequencing (NGS) becoming a part of the diagnostic workflow for these disorders, the detection rate of IBMFS has increased along with the increasing chunk of unclassified cases with no pathogenic variants in any known genes causing IBMFS.
Aims & Objectives: In this study we aimed at analysing paediatric unclassified BMFs by evaluating the variants of uncertain significance, to look for underlying biological processes.
Materials & Methods: All samples were sequenced by a clinical exome panel with an effective depth of 100 $\times$ and analyzed using in-house developed workflows on CLC workbench (Qiagen) and VarSeq (Golden-Helix). Variants were annotated using gnomAD, dbSNP and ClinVar databases and classified based on ACMG classification. 55 samples of Haemolytic anaemia cases that were contemporarily analyzed using the same clinical exome panel were taken as controls to filter out low coverage miscalls and common variants in the population. Biological pathways in unclassified BMFs were analyzed on reactome.org pathway browser.
Result: 66 unclassified paediatric BMF cases with a median age of 8 years (3 months–18 years) and a male-to-female ratio of 2:1 were selected for the study. Most of these cases were further grouped into Pancytopenia (n = 35), Anaemia with thrombocytopenia(n = 14) and Isolated Anaemia(n = 11). On average, 300 unique genes with VUS were identified per case, with some commonly recurring within the groups. To understand the biological processes in these cases, these genes were mapped group wise to biological pathways using Reactome pathway browser. Variants in genes mapping to 'rRNA and tRNA processing in mitochondrion' were seen in 75% of cases of the Pancytopenia group. Variants in the anaemia and thrombocytopenia group were mapped to the 'Interleukin-6 signaling pathway' in 73% of patients, followed by 'FGFR1 and MET signaling pathways' in $\sim$ 50% of the cases. Variants in the isolated anaemia group were mapped to processes related to 'Regulation of RUNX1 expression and activity in 75% of the cases.
Conclusions: In paediatric BMFS cases where pathogenic variants causative of the known IBMFS are absent, variants in genes related to Ribosomal activity, Cytokine signaling, and Transcription Factor pathways should be analyzed. Further functional studies are required to define these as distinct disease entities.

Renal Osteodystrophy: A Conjunction of Hematology, Nephrology and Endocrinology

Farzana Siddiqui, Ashish Gupta, Prakash Singh Shekhawat, Prateek Tripathi, Gaurav Khandelwal

Introduction: Chronic kidney disease (CKD) patients sometimes present with pancytopenia. To evaluate the cause of unexplained pancytopenia, we did bone marrow (BM) examination of those cases and some of them showed the features of renal osteodystrophy (ROD) on morphology. We found four such cases in different age and sex groups and subsequently all of them had increased intact PTH (iPTH) levels. This increase in iPTH causes bone remodeling and secondary myelofibrosis which results in renal osteodystrophy and resultant pancytopenia.
Aims & Objectives: To highlight the need of BM examination in all cases of unexplained pancytopenia in CKD patients, especially with increased iPTH levels.
Materials & Methods: Nine cases of CKD with pancytopenia were included in the retrospective study from Sept 2021 to Aug 2022. Their clinical history and laboratory investigations were analyzed and bone marrow aspiration & trephine biopsies were performed. H&E-stained slides were examined and reticulin staining was done wherever needed.
Result: Bone marrow studies in four out of nine CKD patients with pancytopenia showed features of renal osteodystrophy and myelofibrosis on morphology. They were then tested and found to have increased iPTH levels.
Conclusions: Our study puts an emphasis on the importance of bone marrow examination in pancytopenia evaluation of CKD patients with increased iPTH levels. Renal osteodystrophy is an important cause of pancytopenia in CKD patients. We need a multidisciplinary approach to confirm diagnosis as ROD is an easily treatable disorder which can significantly minimize morbidity and mortality in CKD patients.

Infections and Support Care

A Case of Acquired Pure Red Cell Aplasia Secondary to Parvo B19 in a Middle Age Immunocompetent Patient

Nitish Kumar Patel, K Kgupta, Nilesh Kumar, Swati Singh, Ankita Dewangan

Introduction: Pure red cell aplasia (PRCA) is a rare disorder that presents with anemia secondary to the failure of erythropoiesis. It is characterized by normocytic, normochromic anemia, associated with reticulocytopenia in the peripheral blood and absent or infrequent erythroblasts in the bone marrow. PRCA secondary to parvovirus B19 infection in immunocompetent patient is not common in middle age group.
Aims & Objectives: To present a case of acquired PRCA secondary to Parvovirus B19 in a immunocompetent patient in middle age group.
Materials & Methods: A 36 year female presented with recurrent h/o Generalised body weakness and easy fatiguability for 1 year and had

a history of multiple transfusion requirement,There was no h/o Fever, Joint pain or rashes.
On clinical examination she had Pallor,other systemic examination were within normal limit.Hematological parameters revealed severe anemia with reticulocytopenia and peripheral blood smear showed reduced blood density with predominantly normocytic and normochromic anemia.Bone marrow aspiration showed near absence of late erythroid precursors with predominant early precursors.Bone marrow biopsy revealed picture of mildyhypercelluar for age with loss of late erythroid precursor and morphology of early erythroid precursor was suggestive of Parvo/CMV infection.CMV RT PCR was negative and serology for Parvo viral IgG and IgM trace to be positive.PNH clone was not detected.There was no any immunocompromised status & other investigations were within normal limit.

Patient was treated with IVIG infusion followed by maintenance therapy of cyclosporine and oral steroids.Patient is in follow-up every monthly for last 6 month and doing well.
Result: A case of refractory anemia with diagnosis of acquired PRCA secondary to Parvo B19 in immunocompetent patient in middle age group and use of IVIG followed by cyclosporine and steroid is effective therapy.
Conclusions: In isolated persistent anemia in immunocompetent adults, accompanied by a reticulocytopenia and no other aetiological evidence for the anemia, a parvovirus B19 induced PRCA should be considered.

Incompletely Treated Sputum Negative Miliary Tuberculosis Presenting with Fever and Pancytopenia Diagnosed as Disseminated Histoplasmosis on Bone Marrow Aspiration Study: A Case Report

Purba Jyoti Nath, Balmiki Datta, Chandan Jyoti Saikia, Ena Dowerah

Introduction: Histoplasmosis is a rare fungal disease caused by dimorphic fungi Histoplasmacapsulatum. In India, Histoplasmosis is endemic in Assam, West Bengal and particularly in the Gangetic delta. Many sporadic cases have been found both from North India as well as South India. Like most fungal infections, histoplasmosis is common in immunocompromised patients. In immunocompetent patients, infection is generally asymptomatic and rarely turns into a disseminated form.
Diagnosis is usually made on suspicion from tissue specimen or aspirate cytology from the lymph node, bone marrow, or organ involved showing an intracellular yeast form especially in sections stained with giemsa stain or PAS (Periodic acid Schiff) or Gomorimethenamine silver stain. Culture in Sabouraud's dextrose agar is confirmatory. Antibodies in serum can be detected by CFT and immunodiffusion test can be taken as a supportive evidence.
Aims & Objectives: Here we are presenting a case of incompletely treated sputum negative miliary tuberculosis presenting with fever and pancytopenia diagnosed as disseminated histoplasmosis on bone marrow aspiration study.
Materials & Methods: A 47 year old male presented in the Medicine OPD of FAAMCH with low grade fever, weight loss, cough, decreased appetite, loose stool and worsening of breathlessness for 1 year. He was on antitubercular treatment 6 months before (for radiologically diagnosed milliary TB) and he discontinued the medication thereafter.
clinical examination, he was hypotensive (90/60), with a respiratory rate of 23/min, had pallor and was malnourished with wasting.

On CBC and PBS examination, pancytopenia was noted, Patient underwent bone marrow aspiration cytology.

Bone marrow examination revealed intracellular yeast cells of Histoplasmacapsulatum. On subsequent evaluation, he was found to be immunocompromised and tested positive for HIV.
Result: On laboratory investigations, the LFT was deranged, CBC and PBS examination showed pancytopenia. Bone marrow aspiration cytology showed intracellular (within macrophage) and extracellular yeast forms concluding a diagnosis of HISTOPLASMA CAPSULATUM infection.
Conclusions: Disseminated Histoplasmosis is not an uncommon etiology in patients with fever of unknown origin of most of immunocompromised patients and should always be kept as a differential diagnosis. Bone marrow aspiration may be a diagnostic test for disseminated histoplasmosis cases presenting with pancytopenia and fever of unknown origin.

Predictive Value of Red Cell Distribution Width at Admission as A Marker Of ICU Requirement in COVID-19 Infection

Reema Sachdev, Reeta J Dalal, Vidisha Mahajan, Shanaz Khodaiji

Introduction: COVID-19 is a global pandemic disease first identified in Wuhan, China in late 2019. As of March 2022, over 450 million cases and 6 million deaths have been reported across the world, with the confirmed numbers probably being a fraction of the real numbers. Red Cell Distribution width (RDW) is a measure of anisocytosis, that is, variation in the circulating red blood cell volume. It is a non-specific marker of acute illness. Increased RDW is suggestive of dysfunctional erythropoiesis and/or shortened RBC lifespan. Hence, it is a good predictor of clinical outcome in many disorders.
Aims & Objectives: AIMS: To prove association of RDW with COVID-19 infection requiring ICU stay in a tertiary care hospital.
OBJECTIVES:

Primary Objective: To assess the predictive value of RDW on admission with requirement of ICU as a marker of severity in COVID-19 patients.

Secondary Objectives: To compare RDW to other markers commonly used in COVID-19 infection, such as D-Dimer and CRP.
Materials & Methods: Patients over 18 years of age getting admitted at COVID ward or ICU at P.D. Hinduja Hospital, Mumbai were included in the study. RDW, CRP and D-dimer values of Ward group and ICU group patients were noted and compared.
Result: 234 patients were screened and 190 patients were included in the final study. RDW was found to have significant association with ICU requirement ($p = 0.0066$). Further, an RDW value of 13 or more is found to be 85% sensitive for predicting ICU requirement. RDW of 16 or more is found to be 80% specific for predicting requirement of ICU stay. Regarding secondary objectives, RDW was found to have significant correlation with D-Dimer ($p = 0.0005$) but not with CRP ($p = 0.12$).
Conclusions: RDW can be a potentially useful marker for risk stratification in COVID-19. A value of RDW more than 16 is associated with a significant risk of ICU requirement in COVID-19 disease. Further studies may be indicated to find a statistically significant correlation between RDW values and mortality in COVID-19.

Association of Haemophagocytic Lymphohistiocytosis in Cases of Dengue: A Case Series from Western Rajasthan

Tanya Garg, Khushboo Shripat, Manali Satiza, Siyaram Didel, Abhishek Purohit

Introduction: Haemophagocyticlymphohistiocytosis (HLH) is a potentially life threatening and rare disease which can be either primary (hereditary) or secondary (acquired). The diagnosis is based on

clinical, biochemical and molecular findings. Dengue infection has been increasingly implicated as the cause of secondary HLH. Severe dengue infection complicated by HLH is associated with increased morbidity and mortality. Here, we are reporting a case series of paediatric dengue patients with secondary HLH.

Aims & Objectives: To highlight the importance of keeping high suspicion and early diagnosis of HLH in cases of dengue fever.

Materials & Methods: We are presenting cases encountered in last one and a half year at AIIMS, Jodhpur. Diagnosed cases of dengue infection, admitted to the paediatric IPD with subsequent development of secondary cytopenias were included in this study. Diagnosis of HLH was made on the basis of criteria used in HLH-2004 trial. Laboratory data and clinical details (Haemogram, triglyceride levels, fibrinogen levels, ferritin levels, fever, splenomegaly and presence of haemophagocytosis in bone marrow) were compiled.

Result: During study period, we came across four cases who progressed and developed HLH. Of these, 3 were male and 1 was female with age ranging from 5 months to 17 years. Pancytopenia was seen in one case while the rest three cases showed bicytopenia. Hypertriglyceridaemia and hypofibrinogenemia were seen in all four cases. Ferritin levels were > 3000 ng/mL in all four cases. On physical examination, all four cases had fever, while only two had splenomegaly. On bone marrow examination, all four cases showed prominence of histiocytes with evidence of haemophagocytosis.

The patients were subsequently diagnosed with HLH secondary to dengue infection and were managed with steroids, intravenous immunoglobulins, add-on etoposide, fluid maintenance and symptomatic treatment.

Conclusions: Though uncommon, HLH is a severe complication of dengue infection. Index of suspicion should be high in cases of recurrent or persistent fever, development of cytopenias or extremely high serum ferritin levels as a diagnosis of HLH alters the course of management and heavily impacts the clinical outcome.

Disseminated Histoplasmosis in An Immunocompetent Patient: A Rare Case Report

Souvik Saha, Namrata Kaul, Khaliqur Rehman, Rajesh Kashyap

Introduction: Histoplasmosis is a systemic fungal infection caused by dimorphic fungi Histoplasma capsulatum variant capsulatum and duboisiis. In India, the majority of cases hail from eastern part of country, especially the Gangetic plains. Histoplasmosis can have a varied clinical presentation ranging from asymptomatic infection to progressive disseminated disease. Progressive disseminated Histoplasmosis is usually seen in immunocompromised patients such as HIV, hematological malignancies and post transplant patients. There are very few case reports describing disseminated Histoplasmosis in immunocompetent individuals.

Aims & Objectives: We report one such case of a 40 year old immunocompetent man presenting with disseminated Histoplasmosis.

Materials & Methods: The patient presented with on and off high grade fever for 3 months associated with nausea, vomiting and weight loss. He had received 3 units of PRBC transfusion in 3 months. On examination, he had pallor with hepatosplenomegaly. Complete blood counts revealed pancytopenia. Peripheral blood smear examination showed presence of yeast like Histoplasma in some monocytes. Bone marrow examination was done which also revealed clusters of Histoplasma species both intracellularly in macrophages as well as extracellularly. CT chest and abdomen did not have any evidence of infiltration. He was started on Amphotericin B. His fever subsided and blood counts recovered within 1 week. Peripheral smear 1 week post initiation of therapy did not reveal any Histoplasma species. After 2 weeks of initial intravenous therapy, patient was shifted to oral Itraconazole. He is doing fine with a normal blood count.

Result: Histoplasma requires an acidic damp soil with high organic content for growth. Bats and birds excretions can contaminate the soil and transmit the infection. Our patient lived in an area inhabited by bats. This might explain the source of infection. In immunocompetent individuals, the infection is either asymptomatic or may present with fever, cough and malaise. Disseminated infection involves multiple organ systems like hematologic, CNS, liver, spleen and occurs in immunocompromised patients. Our patient was worked up for any immunocompromised state but nothing significant was found. On treatment, his clinical condition as well as hematologic parameters improved.

Conclusions: Hence, in fever of unknown origin with pancytopenic blood picture a differential of disseminated Histoplasmosis should be kept in mind even in immunocompetent individuals.

Hemophagocytic Lymphohistiocytosis (HLH): Case Series

Sourabh Kumar, Anita Tahlan,Sanjay D Cruz, Monica Gupta, Varsha Gupta

Introduction: Hemophagocytosis is characterized by the presence of red blood cells, platelets, or white blood cells (or fragments of these cells) within the cytoplasm of macrophages. The persistent activation of macrophages, NK cells, and CTLs in patients with HLH leads to excessive cytokine production (cytokine storm) by all of these cell types, and is thought to be responsible for multiorgan failure with high mortality. Instigating trigger for an acute episode is often an infection or alteration in immune homeostasis,mutations at FHL loci & Immunodeficiency syndromes like Chediak-Higashi syndrome, chronic granulomatous disease, etc.Hemophagocyticlymphohistiocytosis(HLH) as per the recommendations from the North American Consortium for Histiocytosis (NACHO)0.1 The terms primary HLH & secondary HLH which have been applied in an attempt to distinguish between an underlying genetic cause versus an alternative source of pathologic immune activation & have been a source of lot of confusion triggered by infections or other immune activating events. Lately gene mutations are being reported in individuals of any age and family history.

Aims & Objectives: Determine possible etiological diagnosis.

Materials & Methods: Retrospective review of cases diagnosed at GMCH-32,chandigarh as HLH and those fulfilling the diagnostic criteria with subsequent bone marrow showing haemophagocytosis are presented in this study Patient age, sex, clinical history, indication for the procedure with clinical & possible etiological diagnosis were analysed.

Result: SEE TABLE.

Secondary causes of HLH commonly include viral infections like EBV, CMV, HIV with bacterial infections causing HLH are less common, with the majority related to Mycobacterium tuberculosis.

Diagnosis of HLH is established: molecular diagnosis consistent with HLH or.

if 5/8 criteria are fulfilled- fever, splenomegaly, cytopenia, hypertriglyceridemia, hemophagocytosis in biopsy, low/absent NK cell activity, hyperferritinemia and elevated sCD25R.

Conclusions: Prompt treatment is critical, but the greatest barrier to a successful outcome is often a delay in diagnosis due to the rarity of this syndrome, variable clinical presentation and lack of specificity of the clinical and laboratory findings.Hemophagocytosis should always be documented as it may be the only clue to underlying conditions. Early suspicion and diagnosis of HLH is essential as it can be life-threatening. An opportunistic infection like Klebsiella though rarely but can be associated with HLH.

Case	Age/Sex	Duration (days)	Hb	TLC	PLT	Clinical diagnosis	Possible etiological diagnosis
1	24/F	45	7.0	3.7	61	Lymphoma/ Infectious mononucleosis	Klebsiella
2	21/F	60	9.0	14.7	139	Disseminated Tb	Brucella
3	55/M	6	8.4	0.6	01	Pancytopenia with HLH	Mycobacterium tuberculosis

A Case Report of Adenocarcinoma with Lepidic Pattern Presenting with Hypereosinophilia

Santa Subhra Chatterjee, Prabuddha Mukhopadhyay

Introduction: Hypereosinophilia as a paraneoplastic syndrome occurs in lung Ca most probably due to increased circulating IL-5 and GM-CSF produced by the tumor cells which stimulate the marrow. Other solid organ malignancies (GI, liver, pancreas, breast, thyroid,GU) and hematological malignancies (malignant lymphoma) also reported to have hypereosinophilia.
Aims & Objectives: To decode a patient of eosinophilia presenting in acutely ill state.
Materials & Methods: A 73 year old male farmer hailing from a village presenting with shortness of breath, clubbing and a swelling over right side of back near armpit.
Result: Initial investigations revealed right sided pleural effusion, leukemoid reaction with severe eosinophilia. The swelling near armpit turned out to be a haematoma. Pleural fluid study revealed exudative effusion which was hemorrhagic with normal ADA and eosinophilia but no suggestion of any atypical cells. CT thorax revealed patchy consolidation without any space occupying lesion. Bone marrow was suggestive of reactive marrow. The diagnosis of adenocarcinoma was clinched on bronchoscopy.
Conclusions: Hypereosinophilia (HE) is defined as an absolute eosinophil count (AEC) $> 1.5 \times 109$/L (or > 1500 cells/microL) in the peripheral blood. Hypereosinophilia as a paraneoplastic syndrome occurs in lung cancer most probably due to increased circulating IL-5 and GM-CSF produced by the tumor cells which stimulate the marrow. Other solid organ malignancies (GI, liver, pancreas, breast, thyroid,GU) and hematological malignancies (malignant lymphoma) also reported to have hypereosinophilia. IL-5 mediates the antitumor cytotoxicity of eosinophils induced by IL-2 against various human tumor cell lines. Nevertheless, hypereosinophilia is generally associated with tumor aggressiveness and poor prognosis.

Multidrug Resistant Organism Associated Sepsis in Neutropenic Patients with Hematological Malignancy: A Cause of Concern

Abhishek Kumar, Prakas Kumar Mandal, Sandeep Saha, Apurba Banerjee,Subham Bhattacharya, Shuvraneel Baul, Rajib De,Tuphan Kanti Dolai

Introduction: Multi drug resistant organisms (MDRO) are a major cause of perturbance in patients with hematological malignancies receiving chemotherapy. MDRO are defined as organisms which are resistance to at least one agent in > 3 anti microbial categories.
Aims & Objectives: To study the patterns of microbial sepsis and their outcomes in patients with hematological malignancies receiving chemotherapy.
Materials & Methods: Cultures were sent for patients with febrile neutropenic (FN) episodes from September 2021 to August 2022 admitted in hematology ward of NRS medical college & hospital. The cultures were analyzed using Bactalert 3D Biomeriuxanalyzer and their antimicrobial sensitivity patterns were studied.
Result: A total of 444 cultures were sent during FN episodes. Majority of these cultures were sent from blood (69.8%) followed by swabs from oral ulcers (13.2%) and Central venous catheter tip (10.2%). Out of which, 23% (105/444) showed growth of different pathogenic microorganisms. The majority of the positive cultures were isolated from acute lymphoblastic leukemia (ALL) 43%(45/105) followed by acute myeloid leukemia (AML) 23%(24/105). Out of all positive isolates, majority (69.91%) were gram negative organisms. Klebsiella species was the predominant isolate (29%), sensitive to Colistin (100%). Methicillin Resistant Staphylococcus Aureus (MRSA) was positive in 23.
Conclusions: Gram negative organism is a major cause of concern in patients with neutropenic sepsis. MDRO constitute 66% of the cases and are the major reason for infection associated mortality in hematological malignancy.

Care of Patients with Haemato-Lymphoid Diseases During Covid: Challenges and Difficulties Faced by Non-Covid Patients in Accessing Health Care

Maria, Bobby Abraham, Reshma, Elsa John, Sruthy V, Roshna P, Jesina Samuel, Priya Prasad, Bonnie A G, Chepsy C Philip

Introduction: Since the national lock down was declared in March in response to the SARS COV-2 pandemic, there has been a concerted exercise to ramp up medical infrastructure and man power, with hospitals devising plans to prepare and allocate resources to organize themselves in managing the emerging cases efficiently.
There was a growing concern that non COVID care and diseases could be affected. Inability to travel, limitation of financial support, lack of support services are among the many reasons that could limit non covid care. There is however a lack of information on this impact.
Aims & Objectives: • To estimate the proportion of patients with benign hematological diseases whose care was affected during covid. • To estimate the proportion of patients with malignant hematological diseases whose care was affected during covid.
Materials & Methods: This was a cross-sectional study conducted among patients under the care of the Regional Advanced Center for Transplantation, Haemato-Lymphoid Oncology and Marrow Diseases (RACTHAM) at the Believers Church Medical College Hospital. Patients visiting the OP service were intervewed face to face. We report on our data collected in the intial phase of lockdown from April 1, 2020 through August 31, 2020.
Result: A total of 505 patients were interviewed and 501 resposnes were recorded. The detailed characteristics are tabulated in Table 1. The majority, 375)74.8%) of patients were diagnosed with non malignat conditions. 256 (50.6%) of the resposndent were male. We noted that only 68 patients (13.5%) could not continue their care during the intial phase of the pandemic and lock down. 156 patients (30.*%) were able to continue their care elsewhere. There were 20 deaths during this period.
Amongst those whose care was affected, 156 transferred care elsewehere. 27 (5.3&) patients were unalble to travel and 15 (2.9%) patients utilised telemedicine dservices to continue their care. ALl patients who required transfusion were able to access the same during this period.

Conclusions: This study provides the first insight to the best of our knowledge on challenges to non covid care by patients with hematological diseases in our region. This study estimating the burden or proportion of patients with hematological diseases whose non covid care was affected has the potential to help in formulating national programs in times of pandemics.

Hemophagocytic Lymphohistiocytosis in Children-Cave of Many Predators-Series of 5 Cases from a Tertiary Care Hospital in Central India

Sana Khatoon, Aastha Paal, Abhijit Choudhary, Richa Juneja, Shikha Jain, Urmila Dahake, Akash bang, Meenakshi Girish

Introduction: Hemophagocytic Lymphohistiocytosis (HLH) is a rare and devastating disorder characterised by uncontrolled immune activation. Incidence of HLH is 1 per 50,000 live births worldwide. Here we present clinical profile of 5 cases of HLH, 2 of primary/familial and 3 of secondary aetiology.
Objectives: To study the clinical profile and laboratory features of a series of HLH cases reported in a tertiary care hospital.
Materials and Methods: A retrospective analysis of case records of 5 children admitted in Department of paediatrics AIIMS Nagpur, diagnosed with HLH between February 2020 to August 2022 was performed.
Results: In these 5 cases HLH was the common initial finding however only 2 were proven as primary HLH on sequencing studies. Out of rest 3- one had Hodgkin lymphoma proven on lymph node biopsy and rest 2 were HLH secondary to infections.
Discussion: Hemophagocytic Lymphohistiocytosis (HLH) is a potentially fatal multisystem hyperinflammatory disorder. (1). Timely diagnosis of HLH is critical to survival and classifying into primary or secondary determines specific therapy and prognostication. Suspected cases with no secondary cause should be evaluated thoroughly and genetic studies must be done to confirm primary/familial HLH. All 5 cases were diagnosed based on the HLH 2008 criteria (2). Hyperferritinemia (ferritin $>$ 500 mcg/L) is easily available marker for the suspicion of HLH particularly in resource limited settings. 3 out of 5 cases of secondary HLH were resolved with treatment of underlying cause however primary HLH was managed with HLH protocol and counselled for bone marrow transplant.
Conclusions: A high index of suspicion, prompt evaluation and early recognition of HLH is vital for survival and a favourable outcome. In a resource limited setting, serum ferritin and bone marrow examination becomes a very useful marker for providing the diagnosis.

Blastomycosis: A Rare Presentation

Sushma Yendamuri, Uday Yanamandra, Naveen Yadhav, Kavitha Bala Anand, A S Menon, Balakrishnan, S P Singh

Introduction: Disseminated fungal infection do occur in immune competent persons.
Aims & Objectives: a rare presentation of disseminated fungal infections.
Materials & Methods: 28 years old young male known case of seizure disorder presented with fever, cough with expectoration for 6 months duration, with significant weight loss with preserved appetite for 4 months and progressive dyspnoea for 1 month (mMRC-4) and midline swelling over neck for 15 days. On clinical examination he was febrile, with tachycardia he was found to have dull note on percussion over Kronig'sisthamus, mammary and axillary region on left side with decreased breath sounds on auscultation. A solitary neck swelling measuring 5 $\times$ 5 cm in size, tender on palpation and firm in consistency, not moving with deglutition and protrusion of tongue and inferior border of swelling was not palpable with negative pemburtons sign. His hemogram showed normocytic normochromic anemia (hb-11.2 gm/dl), biochemical parameters showed A/G reversal and mildly elevated LDH (355 U/L) with normal renal parameters. Tropical infections screening was negative and sputum for MTB was negative. chest x-ray showed homogenous opacity in left lung and CT chest showed Anterior mediastinal mass with doubtful neoplastic etiology. In suspicion of lymphoma bone marrow biopsy was performed which revealed hypocellular marrow with trilineagehematopoiesis with no evidence of lymphoma. FNAC of mediastinal mass was done which showed fibroadipose tissue. Sputum examination on gram stain showed broad based budding yeast, PAS stain and gomorriimethenamine showed thick walled double layered broad based budding yeast present. SDA cultures showed growth with dimorphic fungi (yeast and mold form). CT guided biopsy confirmed yeast forms. His viral markers were negative, immune deficiency panel reveled decreased absolute CD4 and CD8 count with normal immunoglobulin profile. MRI brain showed areas of gliosis with 11 mm sized focal lesion with CSF signal intensity in peripheral part right cerebellar hemisphere. He was managed with antifungals Amphotericin B (1.5–2.5 g) and subsequently transferred to Itraconazole. On follow up he showed symptomatic improvement repeat CT guided Biopsy done to re confirm the presence of fungal elements in otherwise fibrocartilaginous mass.
Result: He was diagnosed as a case of Disseminated fungal infection-Blastomycosis in immunocompetent patient with no neurological involvement.
Conclusions: disseminated fungal infections do occur in immune competent persons.

Applicability of Hscore for Diagnosis of Adult HLH in Indian Population-A Prospective Study

Megha Verma, Nitin Gupta, Jasmita Dass, Vandana Arya, Deepika Gupta, Amrita Saraf, Sabina Langer, Richa Chauhan, Jyoti Kotwal, Ajay Sharma

Introduction: Hemophagocytic lymphohistiocytosis (HLH) is a rare and aggressive clinical manifestation of immune dysregulation, if not diagnosed and treated timely may result fatal course. Gold standard criteria for the diagnosis of HLH is HLH 2004, however non availability of NK cell activity and soluble IL2 levels at most hospitals makes its use difficult. Hscore criteria, has been recently validated in French study for diagnosis of reactive HLH in adults. The parameters included in Hscore are simpler and economically more feasible.
Aims & Objectives: This study was done to assess the performance of Hscore criteria in diagnosis of HLH in adults in Indian population.
Materials & Methods: In this prospective study the clinical data of 120 suspected HLH patients was collected. Utility of the Hscore for diagnosis of HLH in adults, was studied with respect to HLH 2004 criteria and with the expert consensus. The best cut-off value of calculated H-score for diagnosis of HLH, in our set up was studied using Receiver Operating Characteristic (ROC) Curve analysis.
Result: In our study 37 (31%) of 120 patients were diagnosed HLH using HLH 2004 criteria while 65 of 120 (54.5%) patients had score of 3 or 4 out of 6 and due to unavailability of two out of the eight diagnostic criteria, interpretation of these results were difficult.

However, with, H score and expert consensus 63 out of 120 (52.5%), 66 out of 120 (55%) were diagnosed as having HLH respectively. We observed Hscore was highly specific (83.78%) and moderately sensitive (81.82%) for the diagnosis of HLH in adults. Using ROC analysis, we found improved sensitivity (95.3%) and specificity (87.18%) with Hscore cut off value of 145 in our cohort.

Conclusions: Hscore is simple, easily accessible criteria with reasonable diagnostic utility. We recommend calculation of population specific cut off values to obtain optimal utility.

Statistical Test	Hscore(cut off 169) with Expert consensus	Hscore(cut off 169) with HLH 2004	Evaluated Hscore cut off 145 with expert opinion	Evaluated Hscore cut off 145 with HLH 2004
Sensitivity	81.82%	83.78%	95.3%	91.89%
Specificity	94.59%	94.44%	87.18%	88.9%
Accuracy	86.41%	87.27%	92.3%	90.9%

Baseline Peripheral Blood Counts and Outcomes in Patients Presenting with Chinese Virus Infection COVID-19

Rahul Naithani, Preethi Jeyaraman, Pronamee Borah, Omender Singh, Arun Dewan, Nitin Dayal

Introduction: SARS-CoV2 Chinese virus pandemic has significant impact on hematopoietic system.

Aims & Objectives: To report the incidence and pattern of baseline hematological parameters in patients with COVID-19 and their association with severity of disease and outcome.

Materials & Methods: Retrospective observational study.

Result: A total of 440 patients were included in the study. The mean age of the study cohort was 47.5 ± 15.8 years. Fifty percent of patients had at least 1 comorbidity. ICU stay was required in 125 (39.6%) patients. Overall mortality in the study cohort was 3.52%. The average age of patients who died was significantly higher than that of patients who were alive (65.1 years vs 46.5 years; p = 0.000). DM, HTN, CAD and CKD were all associated with higher incidence of ICU stay and mortality. Lymphopenia $< 1 \times 109/\mu l$ was observed in 24.3% and eosinopenia was noted in 44.3% patients. Leukocytosis $> 11 \times 109/\mu l$ was seen in 8.2% of patients. The median neutrophil lymphocyte ratio (NLR) of whole cohort was 2.63. NLR, Lymphopenia, eosinopenia, leucocytosis, D dimer, lactate dehydrogenase (LDH), ferritin and IL6 levels all were associated with need for ICU transfer and mortality. Hemoglobin, red cell distribution width (RDW), PT and aPTT correlated with need for ICU transfer but not with mortality. Ferritin cutoff $\geq$ 751 ng/ml and IL6 levels $\geq$ 64 pg/ml was able to identify all deaths. Ferritin (0.989) and IL-6 (0.985) had very high negative predictive value.

Conclusions: Peripheral blood counts at time of hospitalization is a simple tool to predict outcomes in patients admitted with Chinese virus infection Covid-19.

Table 1. Hematological laboratory findings of patients infected with Chinese Virus on admission and association with outcomes.

	Total	ICU admissions			Mortality		
		Yes	No	P value	Yes	No	P value
Lymphopenia < $1x10^9/\mu l$	107	56	51	0.000	9	98	0.003
Lymphocyte count > $1x10^9/\mu l$	329	69	260		6	323	
Eosinopenia <0.02/($x10^9/\mu l$)	195	78	117	0.000	11	184	0.033
Eosinophil >0.02/($x10^9/\mu l$)	237	47	190		4	233	
Prolonged PT > 13 sec	266	95	171	0.392	10	256	0.437
Normal PT	14	3	11		1	13	
Prolonged aPTT> 35 secs	46	35	11	0.000	1	45	1.00
Normal aPTT	137	40	97		5	132	
Leukocytosis > 11($x10^9/\mu l$)	36	25	11	0.000	5	31	0.000
TLC <11 ($x10^9/\mu l$)	401	100	301		10	391	
Hb< 11 g/dL	67	30	37	0.001	5	62	0.063
Hb> 11g/dL	370	95	275		10	360	

Do Children with Failure to Thrive Have an Altered Immunological Profile?

Saumya Jindal, Richa Gupta, Pooja Dewan, Mrinalini Kotru, Priyanka Gogoi

Introduction: Failure to thrive (FTT) is a commonly encountered problem in pediatric practice referring to failure of expected weight gain, striking lack of well-being and inadequate physical growth. The causes vary according to geographical location/socio-economic status. In developed countries, it is usually a symptom of an underlying disease, while, in developing countries, it is often associated with inadequate caloric intake leading to childhood malnutrition. FTT and malnutrition are closely related as children with FTT are often malnourished. Moreover, malnutrition can manifest as FTT. Some Primary Immunodeficiency Disorders can present as FTT and diagnosis is often missed due to low suspicion. Screening for immunity in such children is critical as unavailability of tests/delay in obtaining results leads to mortality, especially in developing countries. Some literature was found discussing immunity in malnourishment, but none addressed FTT.

Aims & Objectives: To evaluate CBC, percentages of T/B/NK/Naive/Memory Cells and Neutrophil Oxidative Burst in children with FTT and compare with controls.

Materials & Methods: 25 children with FTT (upto 5 years) and 25 healthy age/sex-matched controls were assessed for CBC parameters (Automated Haematology Analyser) and immunological profile (Flow cytometry).

Result: Children with FTT had lower red cell indices including haemoglobin, haematocrit, RBC and MCHC as compared to controls ($p < 0 > p < 0 >$). Total lymphocyte, T/B/NK-cell, Helper T-cell, Cytotoxic T-cell, Naïve Helper T-cell, Naïve Cytotoxic T-cell and Memory Cytotoxic T-cell counts showed no statistical significance. However, CD 45 RO + Memory Helper T-cells were reduced in children with FTT (p = 0.02). Also, the Neutrophil Oxidative Index in DHR Assay showed a significant reduction in cases (p = 0.04). One case showed no change in neutrophil fluorescence after stimulation, suspecting the presence of X-linked chronic granulomatous disease (CGD).

Conclusions: Children with FTT had reduced haemoglobin suggesting possible anaemia. Elevated eosinophil count in such children may be associated with immune deficiency/dysregulation disorders. Decreased Memory T-Cells account for possibly decreased immune response upon repeat antigen encounters, like in HIV infection and decreased Neutrophil Oxidative Burst suggests defective killing of pathogens by phagocytes. Also, the presence of CGD and subsequent infections with Staphylococcus, Salmonella, Aspergillus and Candida species should be suspected in children with FTT.

TABLE. COMPARISON OF PARAMETERS BETWEEN CONTROLS AND CASES

PARAMETERS	CONTROL GROUP	CASE GROUP	STATISTICAL SIGNIFICANCE (P VALUE)
MEAN HAEMOGLOBIN	**10.2 ± 1.53**	**7.78 ± 2.17**	**<0.0001**
MEAN RBC COUNT	**4.38 ± 0.54**	**3.29 ± 1.05**	**<0.0001**
HAEMATOCRIT	**32.38 ± 4.01**	**24.82 ± 6.48**	**<0.0001**
MEAN CORPUSCULAR VOLUME	73.32 ± 6.73	75.69 ± 11.72	0.38
MCH	22.94 ± 2.77	21.36 ± 5.52	0.20
MCHC	**30.65 ± 1.38**	**25.86 ± 5.36**	**<0.0001**
MEAN TLC	9.56 ± 2.94	10.55 ± 3.41	0.27
MEAN NEUTROPHILS COUNT	41.36 ± 14.85	38.4 ± 20.8	0.56
MEAN LYMPHOCYTE COUNT	51.04 ± 15.29	53.84 ± 20.7	0.58
MEAN MONOCYTE COUNT	4.84 ± 2.01	5.12 ± 2.57	0.66
MEAN EOSINOPHIL COUNT	**1.88 ± 1.12**	**5.12 ± 2.57**	**<0.0001**
MEAN BASOPHIL COUNT	0.52 ± 0.5	0.56 ± 0.5	0.77
MEAN PLATELETS COUNT	3.14 ± 0.9	3.05 ± 1.4	0.78
MEAN TOTAL LYMPHOCYTES (CD45+)	49.13 ± 15.9	49.6 ± 19.06	0.92
CD3+ T CELLS (OF TOTAL LYMPHOCYTES)	53.38 ± 13.4	54.86 ± 9.5	0.65
CD19+ B CELLS (OF TOTAL LYMPHOCYTES)	20.71 ± 10.03	21.96 ± 12.52	0.91
CD 16+ 56+ NK CELLS (OF TOTAL LYMPHOCYTES)	13.54 ± 13.36	9.44 ± 7.17	0.40
CD 4+ HELPER (OF CD3+ T)	49.8 ± 10.7	54.18 ± 8.6	0.11
CD8+ CYTOTOXIC T (OF CD3+ T)	36.6 ± 8.0	34.52 ± 9.5	0.40
CD45RA+ NAÏVE (OF CD4+ HELPER T)	60.35 ± 16.49	67.7 ± 13.97	0.09
CD45RO+ MEMORY (OF CD4+ HELPER T)	**28.98 ± 13.25**	**20.82 ± 10.63**	**0.02**
CD45RA+NAÏVE (OF CD8+ CYTOTOXIC T)	67.51 ± 15.47	68.99 ± 14.35	0.72
CD45RO+ MEMORY (OF CD8+ CYTOTOXIC T)	18.46 ± 9.81	19.16 ± 13.27	0.76
STIMULATED NEUTROPHILS %	92.7 ± 8.18	87.51 ± 20.05	0.23
MFI UNSTIMULATED	121640.6 ± 55692.7	8857566.8 ± 4356420.2	0.15
MFI STIMULATED	12759197.6 ± 4468352.7	11990572.4 ± 8250631.5	0.61
MFI INDEX	**132.76 ± 86.14**	**107.69 ± 81.23**	**0.04**

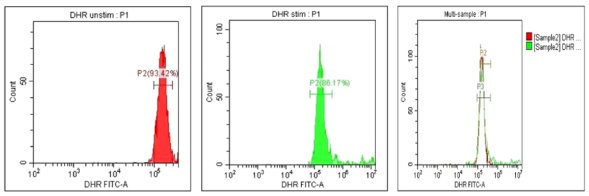


FIGURE. OVERLAPPING UNSTIMULATED AND STIMULATED MFI PEAKS (X-LINKED CGD) IN A CASE

Study of Role of Immature Platelet Fraction as an Early Indicator of Sepsis in Intensive Care Unit

Anshul Vinod Jain, Reema Sachdev, Reeta J Dalal, Shanaz Khodaiji, Vidisha Mahajan

Introduction: Sepsis is a clinical syndrome defined as life threatening organ dysfunction caused by a dysregulated host response to infection. Systemic Inflammatory Response Syndrome (SIRS) is an exaggerated defense response of the body to a noxious stressor (infection, trauma, surgery, acute inflammation, ischemia or reperfusion, or malignancy) to localize and then eliminate the endogenous or exogenous source of the insult. The Immature platelet fraction (IPF) percentage is generally used to differentiate between thrombocytopenia due to bone marrow failure or due to increased peripheral platelet destruction. However, one recent study showed that IPF could predict sepsis occurrence in critically ill subjects. Further, in severe trauma, platelet activation and leukocyte-platelet aggregate formation have been incriminated in the pathogenesis of tissue lesions leading to organ failure. Hence the present prospective observational study hypothesized that platelet activation markers triggered by common infections may help predicting occurrence of sepsis in specific ICU patient populations.
Aims & Objectives: AIM: To determine whether Immature platelet fraction was an nearly indicator of sepsis and could be used in differentiating sepsis from SIRS.
Materials & Methods: A total of 122 patients were enrolled and were divided into two groups of 61 patients each; GROUP 1: Patients admitted in the ICU with fever and proven to have sepsis (confirmed by positive blood culture). GROUP 2: Patients admitted in ICU with fever and having no proven sepsis (blood culture negative).
Result: Mean IPF was found to be higher in group 1 (6.1 + 2.79) as compared to group 2 (3.37 + 2.30) with statistically significant difference when compared using t test.
Conclusions: IPF values obtained within 24 h from ICU admission are higher in patients with sepsis compared to individuals without sepsis and correlate well with the culture report. Since IPF values are higher in febrile patients with proven sepsis than in non-sepsis patients, one can well differentiate these 2 groups. Thus, it can be used as an early marker of sepsis in the ICU setting. The advantage is that it is available along with CBC RETIC on the automated hematology analysers. These patients can benefit from prompt antibiotic therapy which would have been delayed as blood culture reports take at least 24 h to be available for action by the clinician. Therefore, using IPF initially to recognize patients of sepsis, can lead to reduction in morbidity and mortality of these patients.

Hemophagocytic Lymphohistiocytosis in Sepsis: A Prospective Observational Study

Ruovinuo Sachu, Geeta Yadav, Himanshu Dandu, H S Malhotra, S P Verma, Wahid Ali

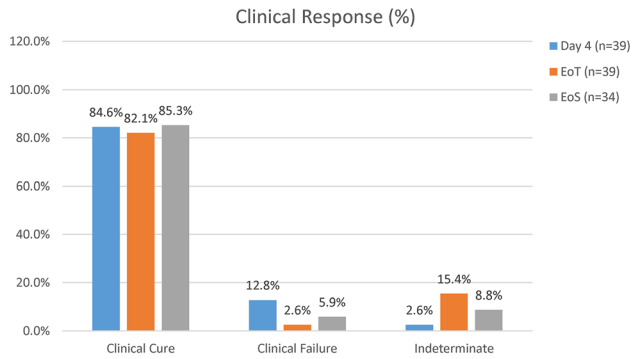
Introduction: Hemophagocytic lymphohistiocytosis, a life-threatening condition is characterized by prolonged and excessive activation of antigen-presenting cells (macrophages, histiocytes), CD8 + Tcells along with the excessive proliferation of T cells and its ectopic migration resulting in a multisystem inflammation. The diagnosis of HLH in adults is difficult and challenging as it has overlapping symptoms and laboratory findings of hyperinflammatory state with sepsis, multi-organ dysfunction, and other disorders of cytokine overproduction. Due to this overlap, its diagnosis is often delayed.
Aims & Objectives: A prospective observational study of the incidence, clinical findings, and outcomes of secondary HLH in sepsis in intensive care units.
Materials & Methods: Patients admitted in the Medicine ICU between 2020 and 2021 with fever, hyperferritinemia (> 500 µg/L), hepatic dysfunction, and or cytopenia were evaluated for the presence of bone marrow hemophagocytosis and were also compared for both HLH 2004 diagnostic criteria and HScore.
Result: Out of the 70 patients included in the study, 39 of them either fulfilled 5 out of the 8 criteria of the HLH-2004 diagnostic criteria or had an HScore of > 169 and were diagnosed as secondary HLH (55.7%). All of them showed evidence of hemophagocytosis in the bone marrow. Of these 39 patients, 22 of them expired (56.4%) while 17 of them recovered and were discharged from the ICUs.
Conclusions: Hemophagocytic lymphohistiocytosis has overlapping features with sepsis leading to its delayed diagnosis and thereby increasing its mortality. Bone marrow Hemophagocytosis was a universal feature seen in all the cases in our study. It is the only morphological evidence of hemophagocytosis. Its early identification and intervention in form of aggressive immunosuppressive therapy are crucial for survival.

A Prospective, Open Label, Single Arm, Multi-Centric, Iis for Evaluation of Efficacy and Safety of Levonadifloxacin for Gram-Positive Bacterial Infection in Immunocompromised Patients

Sudeshna Sen, Bini Mol Thampi, Meera Shabnam

Introduction: Levonadifloxacin is a novel benzoquinolizine antibiotic with rapid bactericidal activity and a unique spectrum of coverage, including multidrug resistant gram positive pathogens, quinolone sensitive gram negative pathogens, atypical and anaerobic pathogens. It has shown potent in vitro activity in eradicating biofilms, has been safely used in patients with renal and hepatic impairment without dosage adjustment and has no risk of myelosuppression or hepatotoxicity.

Aims & Objectives: This study is intended to evaluate the efficacy and safety of Levonadifloxacin for gram positive infections in immunocompromised patients.
Materials & Methods: This Interim Analysis Report captured data of 41 patients receiving levonadifloxacin empirically in treatment of presumed gram positive infections mainly, acute bacterial skin and soft structure infections (ABSSSI), community acquired bacterial pneumonia (CABP) and blood stream infections (BSI), in immunocompromised patients. Total duration of treatment was 5–14 days. Study outcome was clinical success at test of cure ToC visit (7 days after end of treatment). Clinical success in a subject was defined as improvement or resolution of signs/symptoms of the infection to the extent that further antibacterial therapy was not necessary.
Result: Of the 41 evaluable patients, 19 patients received Levonadifloxacin oral therapy, 12 received IV and 10 received IV followed by oral therapy. 39 patients completed therapy and 34 patient follow up data was collected at end of study (EoS). Clinical Cure at end of study was seen in 29/34 i.e. 85.3% patients. No minor/major adverse events were noted during the course of the study.
Conclusions: In the current study interim analysis, Levonadifloxacin was successfully and safely used in the treatment of gram positive infections in immunocompromised patients. Levonadifloxacin could be a preferred bactericidal antimicrobial agent in the management of ABSSSI, CABP and BSI in immunocompromised patients.



Compassionate Drug Access Programs for Refractory/Relapsed Hematological Malignancies: Providing Hope to Those in Despair!

Manik Ghosh, Vinay Anand, Arjin Philips Jacoby, Akshay Lahoti, Saurabh Jayant Bhave, Jeevan K. Garg, Reena Nair, Vivek S Radhakrishnan, Arijit Nag, Mammen Chandy

Introduction: The use of novel immunotherapeutic agents has led to a significant paradigm shift in the management of several hematological malignancies. Most of these agents are not initially available to patients from lower and middle income (LMIC) countries due to significant costs and lack of access. Generosity from pharmaceutical companies allows for compassionate access to these medications to those who do not have access. A compassionate access program (CAP) was launched at Tata Medical Center (TMC), Kolkata with an aim to facilitate access to such medications.
Aims & Objectives: This study aims to assess feasibility and highlight the role of CAPs in providing access to novel therapies in a LMIC.
Materials & Methods: This is an audit of all the applications for access to the medications through the CAP at TMCK. Patient data was collected through previous log entries in hospital records. Graphical representation of data and descriptive analysis was done using Microsoft Excel and SPSS version 26.0.
Result: A total of 122 applications have been approved through the CAP. Patients had a median age of 39 years (Table 1). The beneficiaries of these drugs most commonly lived in West Bengal (68%) and 9.8% of the patients were from outside India. The most common underlying indication was Acute Lymphoblastic Leukemia (ALL) in 26% of the cases, followed by Chronic Myeloid Leukemia (CML—22.1%), Acute Myeloid Leukemia (AML—18.9%) and Classical Hodgkin Lymphoma (CHL—16.4%). Ponatinib was the most common drug (n = 27, 22.1%) obtained through the CAP followed by Brentuximab (18.9%). Through this program, the first infusions of novel agents such as Glofitamab and Mosunetuzumab, was made possible in India. At the time of analysis, 68% (n = 83) of the patients were alive with 11% lost to follow up.
Conclusions: Our data highlights that it is feasible for patients with refractory/relapsed diseases in a LMIC such as India to gain access to novel therapeutic modalities and helps bridge the socioeconomic equity gap in the pharmaceutical and healthcare industry.

Antibody Responses to COVID-19 Infection are Blunted in Hematology/Oncology Patients

Sourav Das, Sushil Selvarajan, Basudev Pokharel, John Roy Thaipadath

Introduction: Coronavirus disease (COVID-19) causes critical illnesses in a large proportion of patients, leading to significant morbidity & mortality. In this regard, the poor prognosis and high mortality rates due to COVID-19 in those with immune dysfunction has been noted. The reason for this has been attributed to poor host immune response in this cohort. However, serological responses have not been well studied in these settings.
Aims & Objectives: To describe the antibody response to COVID19 infections among Immunocompromised patients.
Materials & Methods: In this prospective observational study, antibody responses to SARS CoV-2 in patients with hematological/oncologic conditions requiring chemotherapy, immuno-suppression or post transplantation was studied. Patients included were diagnosed positive between July 2020 to February 2022. Patients were recruited in the study on a follow-up visit where in addition to capturing. Demographics, clinical details and a blood sample was collected for antibody testing against nucleoprotein (anti-N) and spike receptor binding domain (anti-S) (Roche Elecsys ECLIA) platform.

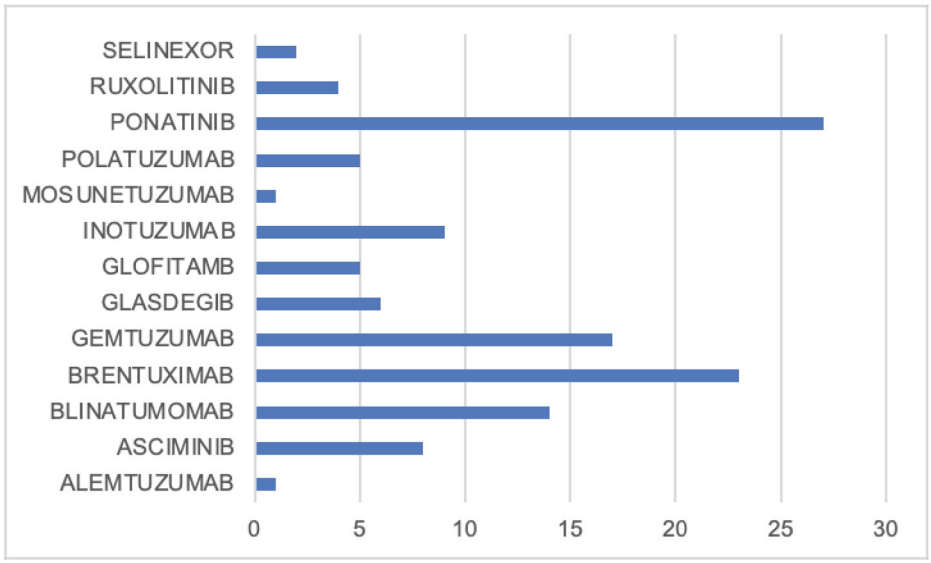
Serological response was studied using chi-square tests, comparing anti-N and anti-S antibody titres across groups.

These were compared against serological response in a parallelly-tested set of healthy control individuals.
Result: A total of 134 immunocompromised patients were included 99(73%) patients on chemotherapy, 19(14%) post- stem cell transplantation and 17(13%) on other immunosuppression. The median age was 24 years with a male predominance (84/134). Of these 97%(130/134) had been vaccinated at time of testing serological response.
Serological response with positive anti -S antibody titres was noted in 88% (118/134) of these patients while only 71% (95/134) showed positive anti -N antibody response. In comparison, among 235 healthy control, positive anti-S antibody responses were found in 97% ($p < 0 >$

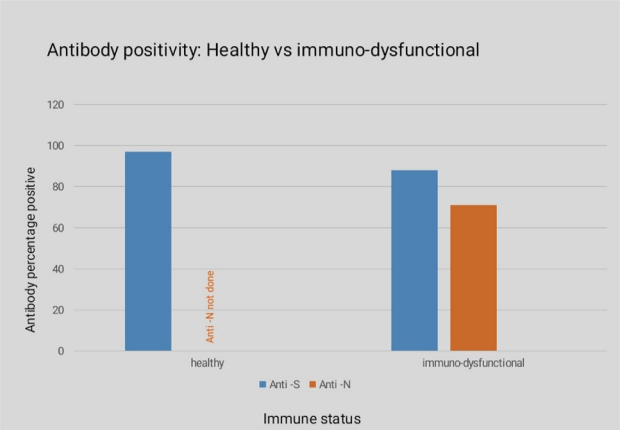
Among the immune-compromised, vaccination(mainly Covishield) also appeared to confer better anti-S antibody responses in comparison to those un-vaccinated prior to COVID-19 infections, although the difference was not statistically significant (94.4 vs 86.6, $p = 0.348$).
Conclusions: This is the first study that has prospectively documented serological response to COVID-19 infection in patients with underlying hematology/oncology disorders. Overall a pattern of inferior antibody response to Sars Cov2 was observed in this immunologically dysfunctional cohort, as compared to a healthy control set.

Table 1 Baseline characteristics of this patient cohort and distribution of CAP medications

No. of patients	122	
Median age in years (range)	39 (21-55)	
Male: Female	76:46	1.65
Place of origin		
West Bengal (%)	82 (67)	
India (apart from WB) (%)	27 (22)	
Outside of India (%)	13 (11)	
Outcomes		
Alive (%)	83 (68)	
Dead (%)	28 (23)	
Lost to follow up (%)	11 (9)	
Indication for drug access		
ALL (%)	32 (26.2)	
AML (%)	23 (18.9)	
Beta Thalassemia	3 (2.5)	
CHL (%)	20 (16.4)	
CML (%)	27 (22.1)	
FL (%)	1 (0.8)	
MM (%)	2 (1.6)	
Drugs accessed through CAP		



CSA, cyclosporine A; ETP, eltrombopag; ATG, anti thymocyte globulin; BMT, bone marrow transplant



T Cell Dysfunction as a Potential Contributing Factor in Post-COVID-19 Mucormycosis

Geeta Yadav, Himanshu Dandu, Hardeep Singh Malhotra, Manish Kumar, Prashant Gupta

Introduction: The second wave of COVID-19 in India was followed by large number of mucormycosis cases. Indiscriminate use of immunosuppressive drugs, underlying diseases like diabetes cancers, or autoimmune diseases was thought to be the cause. However, the mortality was not as high as that seen in non-COVID mucormycosis.
Aims & Objectives: To study the detailed characteristics of T-cells for evaluating the underlying differences in the T-cell immune dysfunction in post-COVID and non-COVID mucor patients.
Materials & Methods: The study included histopathologically confirmed cases of mucor (13 post-COVID, 13 non-COVID) and 15 healthy individuals (HI). Expression of T-cell activation (CD44, HLADR, CD69, CD38) and exhaustion (CTLA, PD-1, LAG-3 and TIM-3) markers was evaluated by flow cytometry.
Result: All cases showed significant depletion of T-cells compared to HI. Both post-COVID and non-COVID groups showed increased activation and exhaustion as compared to HI. Non-COVID mucor group showed significant activation of CD4 + T cells for HLADR and CD38 ((P = 0.025, P = 0.054) and marked T-cell exhaustion in form of co-expression of PD-1 and LAG-3 on both CD4 + and CD8 + T cells in comparison to post-COVID patients (P = 0.002, P = 0.001). Additionally, co-expression of PD-1 & CTLA and LAG-3 & TIM-3 on CD8 + T cells was statistically significant in non-COVID mucor patients ((P = 0.031, P = 0.003).
Conclusions: Immunosuppression in non-COVID mucor showed pronounced exhaustion of T-cells in comparison to post-COVID mucor cases implicating T-cell immune dysfunction is much more severe in non-COVID mucor which are in a state of continuous activation followed by extreme exhaustion leading to poorer outcome.

Analysis of Etiology of Mortality in Hematological Malignancy from Hematology Ward of a Tertiary Health Care Center

Abhishek Kumar, Rajib De, Apurba Banerjee, Shipla Roy, Sandeep Saha, Subham Bhattacharya, Shuvraneel Baul,Tuphan Kanti Dolai

Introduction: Hematological malignancies contribute maximum mortality in hematology ward. Mortality depends on multiple factors i.e. patients' general condition, type of malignancy, phase of chemotherapy, availability of resources etc.
Aims & Objectives: To evaluate the mortality rate & etiology of mortality in patients with different hematological malignancies admitted in hematology ward of NRS hospital, Kolkata.
Materials & Methods: Retrospective data of all the patients who expired in hematology ward of NRS hospital from January 2020 to August 2022 was documented. Mortality Data was categorized in to type of malignancy, phase of chemotherapy and etiology of mortality.
Result: Between January 2020 to August 2022, a total of 14.05% (176/1252) patients expired out of total admission in hematology ward. Out of 1252 admissions, 992(79.2%) cases were of malignant disease in which mortality was 16% (161/992) and 260(20.8%) cases were of benign disease in which mortality was 5.7% (15/260). Out of total admitted malignant disease, (510/992)51.4% were ALL in which mortality was 15% (77/510), (260/992)26.2% were AML in which mortality was 24.6%(64/260), (150/992)15.1% were Lymphoma in which mortality was 10%(15/150) and (72/992)7.3% were Other hematological malignancies with mortality of 6.9%(5/72). During above mentioned time period, Among total ALL (512) cases, 300 cases received Induction therapy, 185 received consolidation therapy and 25 cases were relapse disease. Among total ALL admissions, Induction mortality was 14.6%(44/300), consolidation mortality was 4.3%(8/185) and relapse mortality was 100% (25/25). Among total AML (260) cases, 160 cases received Induction therapy, 95 cases received consolidation therapy and 5 cases were relapse disease. Among total AML (260) cases, Induction mortality was 30%(48/160), consolidation mortality was 11.5%(11/95) and relapse mortality was 100%(5/5). The overall major cause of mortality was sepsis 85.2% (150/176) followed by pneumonia 5.1% (9/176).
Conclusions: The Mortality rate (16%) was very high in hematological malignancy. Mortality was highest in Relapse disease (100%) of ALL and AML followed by induction (30%) and consolidation (11.5%) phase respectively. Sepsis (85.2%) was the leading cause of mortality.

GRAIN Study: (Granulocytes In Infections)—Use Of Granulocyte in Haemopoietic Stem Cell Transplant

Karthik Rengaraj, Steven Lionel,Sharon Lionel, Fouzia N A, Anup Devasia, Aby Abraham, Kavitha Lakshmi, Alok Srivastava, Vikram Mathews, Biju George, Anu Korula Dolly Daniel,Sushil Selvarajan,Uday Kulkarni

Introduction: Granulocyte transfusions (GTx) are used to combat infections in neutropenic patients despite conflicting evidence. In severe sepsis granulocytes augment neutrophil function resulting in resolution of infection along with appropriate antimicrobial therapy. However immune mediated off target effects, especially in a transplant setting may be detrimental. Hence use of GTx either as pooled buffy-coat preparation or G-CSF and steroid mobilised apheresis product was studied.
Aims & Objectives: We aimed to analyse the indication, dose, duration of GTx and its effect on infection.
Materials & Methods: During January 2020-December 2021, 299 allo and 162 auto transplants were done. All transplants using GTx during peri-transplant period (conditioning till Day 100) were analysed. Indication (Empirical or documented infection), median number of days of GTx, total number of bags pooled for buffy-coat, median neutrophil cell doses in the final buffy coat and granulocyte apheresis product were collected from blood bank. The number of patients who developed acute respiratory decompensation in 24 h following GTx was retrieved from clinical records. Infections during the peri-transplant period and number of days to clearance was analysed. Overall survival for patients receiving GTx was compared with mean granulocyte cell dose. Data was analysed using SPSS software.
Result: Baseline characteristics and GTx dosage in table 1.
There were 97 blood cultures positive in 73 patients (65%), and other documented bacterial infections were urine in 19 (17%), skin and soft tissue in 16 (15%), stool in 10 (9%) and sputum in 17(16%). Blood

cultures cleared in 81% of patients while only 28% had documented clearance of other cultures. 44 Patients (39%) had probable fungal pneumonia with positive galactomannan and radiological evidence while 70 (63%) had possible fungal pneumonia. Documented clearance with galactomannan negativity or resolution on HRCT occurred in 28 (25%). Invasive fungal sinusitis or otitis was seen in 8 patients each with clearance observed in 50%. OS was 48.2% and higher (56.6% vs 40.7%, P = 0.161) in patients receiving cell dose of $> 1 \times 10^{10}$/L.

Conclusions: Despite ease of availability of pooled buffy coat, higher dosage of granulocyte per product may be more efficacious to combat infection and needs to be targeted. Use of GTx did not affect engraftment hence can be used safely in the peri- transplant period.

Table 1.Description of HCTs requiring granulocyte/buffy (N= 112/461)

Median Age	28 years	Range (1 – 63 years)
Gender	63 Males (56%)	49 Females (44%)
Matched Related Transplant Haploidentical Transplant Autologous Transplant	46/189 Patients (41%) 48/110 Patients (43%) 18/162 Patients (16%)	43 Full match, 3 one gene mismatch
Diagnosis for Allogenic transplant (n=94)	Aplastic Anaemia AML ALL Others	31 patients (33%) 29 patients (31%) 20 patients (21%) 14 patients (15%)
Diagnosis for Autologous transplant (n=18)	Lymphoma Multiple Myeloma Others	12 Patients (66%) 4 Patients (22%) 2 Patients (11%)
Number of tra- splant	First transplant Second transplant	84 Patients (89%) 10 patients (11%)
Patients with underlying malignancy (67 patients)	CR 1 CR 2 PR or less	32 patients (48%) 24 patients (36%) 11 patients (16%)
Conditioning regimen	Myeloablative RIC/NMA	51 patients (45.5%) 61 Patients (55.5%)
Median CD34 Cell dose	9.04 X 10^6cells/kg	Range 2.35 to 22 X 10^6cells/kg
Median days to Engraftment	Neutrophil Engraftment Platelet Engraftment	14 days 20 days
Incidence of Acute or Hyperacute GVHD	19 patients (17%)	Grade I/II – 68% Grade III/IV – 32%
Granulocyte transfusions (GTx)	Pooled Buffy coat only Apheresis product only Both	70 Patients (63%) 2 Patients (1%) 40 Patients (36%)
Median Number of days of GTx Median Number of bags pooled	4 days 4 Bags	Range 1- 26 days Range 1-6 bags
Median Cell dose	Pooled Buffy Coat Apheresis Product	0.98 x 10^{10}/ L 1.34 x 10^{10}/L
Higher Cell Dose >1 x 10^{10}	Pooled Buffy Coat Apheresis Product	53/110 patients (48%) 23/42 patients (55%)
Respiratory distress post GTx	46 patients (41%)	

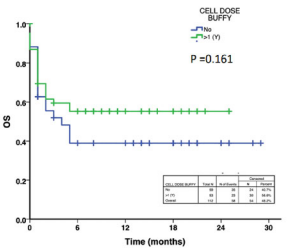


Fig 1. Overall Survival Curve in those receiving higher granulocyte cell dose.

Lymphoma and Myeloma—Clinical

Uncommon Presentation of Multiple Myeloma

Subhash Yadav, L P Meena, Arun Kumar Singh, Ravichand, Atique, Bhargav

Introduction: Multiple myeloma is a malignant plasma cell disorder. Though it commonly presents with anemia, bony lytic lesions, hypercalcemia and renal involvements. It is diagnosed only with high clinical suspicion and if presenting with uncommon presentation is like discovering a needle in a haystack.

Aims & Objectives: To report uncommon presentation of multiple myeloma with massive splenomegaly.

Materials & Methods: 51 year male presented with generalised body weakness, fatiguability, mild fever and dragging sensation per abdomen for one and a half months.

On clinical examination he had pallor and massive splenomegaly. Haematological parameters showed pancytopenia and hypergammaglobulinemia.

X-ray skull showed multiple lytic lesions. Massive splenomegaly seen on USG. rK 39 was negative. Serum total IgM was normal.

Protein electrophoresis showed M band of 2.1 gm/dl in gamma region, Immunofixation showed IgG lambda. Bone marrow aspiration showed sheets of plasma cells (80%) and on immunohistochemistry plasma cells positive for CD138 and lambda restriction.

Patient was initially given steroids and later Bortezomib, Lenalidomide low dose (10 mg/kg), Dexamethasone regimen was continued. Patient gradually showed improvement in pancytopenia and spleen regressed from 21 to 15 cm after 4 months of therapy.

Result: Patient was diagnosed as a case of multiple myeloma and showed improvement after treatment with steroid, bortezomib, lenalidomide.

Conclusions: The actual clinical presentation in multiple myeloma is highly variable. Though splenomegaly is a feature of Waldenstrom macroglobulinemia, multiple myeloma may present with extra-medullary manifestations like splenomegaly. Early diagnosis in such cases is important to prevent morbidity and mortality.

Conjunctival Lymphoma: A Case Report

Prerna Pramanik, Maitreyee Bhattacharyya

Introduction: Conjunctival lymphoma is an ocular malignancy which is derived from the clonal proliferation of lymphocytes. Conjunctival lymphoma accounts for only 5–10% of all extranodal lymphomas. Majority of conjunctival lymphoma is extranodal marginal zone lymphoma (80%), followed by follicular lymphoma (8%),diffuse large B-cell lymphoma (3%) and mantle cell lymphoma (3%) and rarely T and NK cell subtypes. Conjunctival lymphomas may appear as isolated neoplasm or a part of the systemic disease.

Aims & Objectives: To evaluate a case of primary conjunctival lymphoma and to discuss the etiology, diagnosis and treatment of the same.

Materials & Methods: We report a case of 40 year old man who presented to the Haematology OPD for the evaluation of a slowly growing painless swelling over his right eye conjunctiva with increased lacrimation. There were no systemic symptoms and normal systemic examination. Patient underwent conjunctival biopsy.

Result: Histological and immunohistochemical examination of the conjunctival biopsy led to the pathological diagnosis of extranodal marginal zone lymphoma of mucosa associated lymphoid tissue (MALT-lymphoma). Patient was then referred to the Radiotherapy Department and was started on Involved Field Radiotherapy.

Conclusions: Conjunctival MALToma though rare is completely responsive to therapy and has a good prognosis. High grade clinical suspicion is required for prompt diagnosis and treatment.

Secondary Histiocytic Sarcoma in Pre-B-Acute Lymphoblastic Leukemia: A Rare Case Report

Abhishek Das, Sharanya Ramakrishna, Vivek Radhakrishnan, AaishwaryaDhabe, Mayur Parihar, Lateef Zameer, Neeraj Arora, Reena Nair

Introduction: Only handful cases of histiocytic sarcoma (HS) in adult acute lymphoblastic leukemia (ALL) patients have been reported in the literature.

In this case report, we discuss a case of a lady with pre-B-ALL on maintenance treatment who developed extensive HS.

Aims & Objectives: A Retrospective analysis of presentation and outcome of a rare cancer.

Materials & Methods: Retrospective analysis of clinical, laboratory and radiological data retrieved from Electronic medical records.

Result: Case report of a 37-year-old female presented with dysmenorrhea, backache and bilateral lower limb swelling for one week. CT of abdomen showed.

homogenously enhancing soft tissue density retroperitoneal mass encasing the aorta and IVC, bulky uterus, ascites, and a bulky solid right adnexal space occupying lesion. Biopsy from the adnexal mass was performed and immunostaining showed tumor cells to be strongly and diffusely positive for TdT and CD20 and negative for CD3, chromogranin, synaptophysin and desmin. Bone marrow (BM) examination revealed 70% blasts and cytogenetic analysis revealed complex karyotype (> 3 abnormalities) which also included TP53 deletion She was categorized as high-risk Pre-B-ALL and was administered BFM chemotherapy protocol. Following induction and intensification, the patient's BM showed complete response. On maintenance therapy, the patient presented with new onset left sided limp. MRI of pelvis was suggestive of focal erosions of the left iliac blade with a large collection in the left iliac fossa. A core biopsy of the left iliac bone showed sheets of atypical oval to spindle cells with abundant eosinophilic cytoplasm with fine vacuoles and pleomorphic vesicular, ovoid to elongated nuclei diffusely positive for CD 163, CD45, CD68, OCT-2 and negative for CD43, Ki-67—60%, S-100 &

CD 1a negative, negative for TdT and CD34 with no intervening reactive cells thus favoring a diagnosis of a secondary histiocytic sarcoma with no signs of ALL relapse.PET scan showed disseminated metabolically active ultiple visceral deposits in lung, pleura, peritoneum, tail of the pancreas, uterus, metabolically active parietal deposits in anterior abdominal wall, right gluteal region,right arm, metabolically active internal mammary, retrocrural and pelvic lymph nodes, metabolically active periarticular deposits involving both hip joints and metabolically active large expansile lesion in left iliac blade She was initiated on salvage chemotherapy with toposide, cyclophosphamide, and dexamethasone for one month following which she developed severe urinary tract infection with sepsis requiring intensive care management. Patient and family opted for best supportive care and palliative treatment. Subsequently, the patient succumbed to disseminated HS.

Conclusions: In the setting of an ALL patient it is important to be aware and consider possibility of HS as one of the differentials. As there is no standard protocol for treatment of HS, and it has a poor prognosis. Therefore, active reporting of such cases needs to be emphasized to better comprehend the time and pattern of occurrence, incidence as well as molecular pathology.

Frequency of Bone Marrow Involvemen in Elderly Patients with Non-Hodgkin's Lymphoma with Primary Lymph Node Involvement

Victor Tomacinschii

Introduction: Non-Hodgkin's lymphomas (NHL) are malignant tumors that develop from lymphoid tissue. They can affect various organs and tissues, but the most common primary localization is the peripheral lymph nodes (LNs). Bone marrow testing (BM) is considered indispensable for assessing and staging NHL at the time of initial diagnosis as well as response to therapy. The study of CM can provide important diagnostic and prognostic information in patients with NHL.

Aims & Objectives: We aimed to determine the incidence of bone marrow involvement in elderly NHL patients with primary lymph node involvement.

Materials & Methods: A retrospective study of 78 NHL patients with primary LN lesion aged 60 to 84 years was conducted to determine the relationship between the primary lesion and the frequency of bone marrow involvement.

Result: NHL more often developed predominantly in peripheral LNs (84.7%), less often in mediastinal LNs (6.4%) and abdominal LNs (8.9%) (Table 1). Aggressive NHL prevailed (59.0%), and indolent NHL was diagnosed in 41.0%, more often with lesions of the cervical lymph nodes (47.4%), inguinal lymph nodes (41.7%) and abdominal lymph nodes (42.9%). BM metastasis occurred in 43.2% of cases, confirmed through BM biopsy. It should be emphasized that CM lesions occurred with approximately the same frequency in patients aged 60 to 70 and 71 to 80 years (42.3% and 44.4%, respectively) (Fig. 1), and occurred mainly with indolent NHL (17 out of 32 patients followed up to stage IV—53.1%). In contrast to indolent NHL, in aggressive variants, the bone marrow was affected in only 2 of 46 patients. BM metastases were more common in patients with NHL in the abdominal, axillary, and cervical lymph nodes (66.7%, 66.7%, and 55.5%, respectively). In primary lesions of the inguinal and supraclavicular lymph nodes, bone marrow involvement was detected less frequently (28.6% and 16.7%, respectively).

Conclusions: NHL with primary LN involvement was more common in people aged 60 to 70 years. The defeat of the CM was determined in 43.2% and more often diagnosed in indolent NHL—53.1% compared with aggressive NHL—4.3%. The most common areas of lymph nodes associated with CM lesions were axillary (66.7%), abdominal (66.7%), and cervical (55.5%).

Blastoid Variant of Mantle Cell Lymphoma of Tonsil: An Uncommon Case

Ashwini Narayankar, Ashwini Narayankar, Sharanya Ramakrishnan, Vivek Radhakrishnan, Neeraj Arora, Lateef Zameer, Rimpa Achari, Reena Nair

Introduction: Mantle Cell Lymphoma (MCL) typically has a nodal involvement, but extra-nodal sites can be involved. Most common extra nodal sites include gastrointestinal tract, bone marrow, liver, and spleen. Isolated involvement of Waldeyer's ring is rare. Majority of the case reports/series describing isolated involvement of waldeyer's ring are indolent MCLs.

Aims & Objectives: We present a case of a patient diagnosed with blastoid tonsillar MCL, an uncommon presentation.

Materials & Methods: Details regarding clinical presentation, histopathology, radiographic findings, management were taken from patient's Electronic medical record (EMR) at our center.

A 50-year old male presented with a history of foreign body sensation in throat for a period of two months, not accompanied with fever, weight loss or anorexia. Clinical examination revealed an enlarged right tonsil with no cervical lymphadenopathy. The patient underwent right tonsillectomy. The histopathological features were consistent with the diagnosis of blastoid variant of MCL. The patient's peripheral blood smear and bone marrow examination was unremarkable. A PET-CT revealed post-operative healing changes in the right tonsillar fossa (diffuse FDG uptake) with no definitive evidence of active malignant disease.

Result: The tonsillar MCL is often indolent with absence of SOX-11 and a low Ki-67. Unlike, majority of tonsillar MCL reported in literature, our patient had a blastoid morphology and was positive for SOX-11 with high Ki-67 ($\sim$ 90%). This implied, we were dealing with localized disease yet an aggressive variant. Based on multidisciplinary recommendations and considering the young age, localized/limited stage disease (Stage 1AE-tonsil) with post-tonsillectomy status, despite the aggressive nature, we decided to administer a non-cytarabine based regime followed by IFRT consolidation and defer High dose chemotherapy and HSCT. He received 4 cycles of RCHOP chemo- immunotherapy [Rituximab, Cyclophosphamide, Hydroxydaunorubicin hydrochloride, Vincristine and Prednisone], followed by consolidation with intensity modulated involved field radiotherapy (IFRT) to right tonsil and right neck (36 Gy 20# for 4 weeks). The patient is on follow-up since last 4 years and is in clinical and radiological remission till date.

Conclusions: Primary blastoid variant MCL of palatine tonsil, continues to be a clinical challenge, therefore management must be done with multidisciplinary co-operation. Moreover, "how much is too much" must be decided based on clinical judgment for a limited stage disease even in a young, fit individual.

Neutropenia Secondary to Indolent Gamma Delta (??) T Cells Proliferation

Hari Neupane, Sudhanshi Raina, Shruti Madan, Anand Balakrishnan Deepesh Lad Sreejesh Sreedharanunni

Introduction: Normal gamma delta ($\gamma\delta$) T-lymphocytes have the morphology of large granular lymphocytes and express Pan-T antigens and NK-associated antigens along with cytotoxic molecules like perforin and granzyme B. $\gamma\delta$ T-cell leukemia/lymphomas are very uncommon and include four types, namely, $\gamma\delta$ T-cell acute lymphoblastic leukemia/lymphoma (T-ALL), $\gamma\delta$ T-cell large granular

lymphocytic leukemia, hepatosplenic T-cell lymphoma, and skin and mucosal γδ T-cell lymphoma. In addition, γδ T cell proliferations are associated with several infections and immunological disorders. Here we report a patient with γδ T-cell proliferation in a patient presented with peripheral blood leukopenia and splenomegaly.

Aims & Objectives: .

Materials & Methods: .

Result: 48 years male presented with complaints of fever and abdominal discomfort for 20 days. On examination, there was no significant physical findings except for hepatomegaly and moderate splenomegaly. The lab investigation showed persistent leukopenia (1800/micL) and severe neutropenia (209/micL). With the suspicion of myelodysplastic syndrome, autoimmune disorders, or lymphoma/leukemia, a bone marrow examination was performed. It was hypercellular with adequate representation and normal maturation of erythroid precursors and megakaryocytes. There was a left shift in the myeloid series. There was no interstitial or sinusoidal excess of lymphocytes and blast, or any dysplasia. FISH testing for -5/5q, -7/7q, + 8, and 20q were negative. A flow cytometry and TCR gamma re-arrangement assay revealed clonal γδ T cells proliferation. A final diagnosis of neutropenia secondary to indolent clonal γδ T cell proliferation was made.

Conclusions: Abnormal T cell clones may be present in a patient with cytopenia and demand a high level of suspicion when microscopic evaluation of bone marrow aspirate fails to explain the same. Abnormal immune phenotype can be seen in patients with reactive T cell proliferation and flow cytometry alone may not solve the doubt. Hence, a holistic approach (clinical features,

morphology, immune-phenotype, cytogenetic and molecular findings) is sometimes needed for accurate diagnosis.

Clinical, Laboratory Profile, Treatment and Outcome Of Primary Amyloidosis in the Era Of Novel Agents: An Experience from a Tertiary Care Hospital

Yogalakshmi Sivaprakasam, Jyoti Kotwal, Pallav Gupta, Deepika Gupta, Nitin Gupta

Introduction: Diagnosis of AL amyloidosis requires demonstration of amyloid in affected tissues along with clonal plasma cells in bone marrow or presence of monoclonal light chains in blood. With the availability of serum light chain assay and immunophenotyping by flow cytometry, more cases of AL amyloidosis are being diagnosed. Here we present our experience of AL amyloidosis diagnosis and treatment in the era of modern diagnostics and therapy.

Aims & Objectives: We aimed to describe the clinical presentations, laboratory features, treatment and outcomes of patients with AL amyloidosis in a single center using standard diagnostic tests and treatment with novel agents.

Materials & Methods: A retrospective analysis of AL amyloidosis patients, diagnosed in our hospital, a tertiary care center from January 2016 to June 2022 was conducted. The data was collected from departmental database. All statistical analyses were done by SPSS version 17.

Result: Diagnosis of AL amyloidosis was done in 31 patients. Median age of presentation was 61 years. 25 (80.6%) were males. Major symptoms were pedal edema (38.7%) and shortness of breath (32.3%). Twenty four (77.4%) presented with ECOG PS $\geq$ 2. Most common systems involved were cardiac (54.8%) and renal (54.8%). Fourteen (45.2%) had two or more systems involvement while 17 (54.8%) had single system involvement. Lambda monoclonal light chain was present in 83.9% and kappa monoclonal light chain in 16.1%. Median M-protein was 0.59 g/dL (range 0–2 g/dL) and median bone marrow plasma cells were 6% (range-1–18%). Eighteen patients were treated; cyclophosphamide, bortezomib and dexamethasone (CyBORD) in 12/18 (66.7%) and bortezomib + dexamethasone in 6/18 (33.3%). Among 18 patients followed up with median follow up of 9 months (range 1–64 months), six expired; three due to COVID, two due to cardiac arrhythmia (during first cycle) and one due to relapse and rest 12 were alive. Among the 12 patients who were alive 6 were in complete hematological response.

Conclusions: Our study presents the spectrum of clinical manifestations, management and outcomes of primary amyloidosis in Indian context. There is a need to increase the awareness among the physicians about amyloidosis so that early diagnosis can be made and timely treatment can be done with novel agents to improve the dismal historical results.

CYBORD as First Line Therapy in Transplant Eligible Newly Diagnosed Patients of Multiple Myeloma: Single Centre Study

Deepak Rajendra Patil, Nirali Chandan,Shashikant Apte, Kannan Subramanian, Rajesh Phatale, Chandrakant Lahane, Abhijit Giram, Shubh Purohit, Digambar Panchal

Introduction: Lenalidomide, bortezomib, and dexamethasone followed by autologous stem cell transplantation is recognized as standard frontline therapy for transplant-eligible patients in newly diagnosed myeloma patients. However present study aims to evaluate the outcomes in patients treated with CyBorD as induction regimen in preparation for subsequent ASCT.

Aims & Objectives: To study response of CyBorD as first line therapy in newly diagnosed transplant eligible multiple myeloma patients in our institution.

Materials & Methods: Retrospective analysis of 24 patients in last 4 years who were treated with CyBorD as first line therapy after diagnosis of myeloma.

Induction consisted of four 21-day cycles of Cyclophosphamide 300 mg/m2 on days 1, 8, and 15; bortezomib 1.3 mg/m2 on days 1, 4, 8, and 11; dexamethasone 40 mg on days 1, 8, 15, 22.

The demographic profile, disease characteristic and outcomes are presented below.

Result: Out of 24 newly diagnosed multiple myeloma patients, median age 58 years, who were administered 4 cycles of CyBorD as first line therapy, 3 patients achieved stringent complete response(13%), 4 patients achieved complete response(17%), 7 patients achieved very good partial response(29%), and 7 patients achieved partial response(29%).

2 patients had stable disease(8%) and 1 patient had a progressive disease(4%).

Only one patient had Grade 3 Bortezomib induced peripheral neuropathy.

11 out of 14 patients (3 sCR, 7 CR, 4 VGPR) underwent autologous stem cell transplant post 4 cycles of CyBorD as consolidation.

Conclusions: 59% patients (sCR + CR + VGPR) underwent autologous stem cell transplant post induction by 4 cycles of CyBorD.

CyBorD is an effective, relatively inexpensive, safe and easy to administer therapy in Multiple myeloma patients. Therapy related complications are negligible and can be used for induction in transplant eligible multiple myeloma patients.

Mantle Cell Lymphoma: A Single Centre Experience from a Tertiary Care Centre in North India

Poorvi Kapoor, Faheema Hasan, Sanjeev, Rajesh Kashyap

Introduction: Mantle cell lymphoma (MCL) is an aggressive lymphoma with an incidence of 2.4–6% among all non-Hodgkin's lymphoma. Though with the addition of rituximab to standard

chemotherapy backbone with autologous stem cell transplant (ASCT) consolidation and novel small molecule inhibitors, the outcome of MCL has improved, however, not many patients undergo ASCT due to financial constraints.

Aims & Objectives: To study the clinical profile of all patients of MCL over a period of 6 years and assess their outcome.

Materials & Methods: This study was a retrospective cohort study which included all patients diagnosed with mantle cell lymphoma, between January 2016 to January 2022, conducted in the Department of Haematology, Sanjay Gandhi Institute of Medical Sciences, Lucknow, India. A total of 53 cases were included in the study. SPSS-23 was used for the data analysis.

Result: The median age was 59 years (ranging from 39–81 years), with a male to female ratio of 5.3:1. The ECOG performance status was of 0–2 was seen in 85.2%. The median haemoglobin, leukocyte count and platelet count at presentation was 10.6 g/dL, 7400/mm3 and 1,52,000/mm3 respectively. Of the 53 patients, 48% presenting with B symptoms. The median Lactate dehydrogenase levels were 521 (ranging from 220–1230). 72% patients presented with stage IV disease and MIPI score was high, intermediate and low risk in 43.4%, 32.1% and 24.5% low risk. 48% patients received RCHOP/RDHAP regimen, 36% received RCHOP and 16% received R-Benda. 5 patients underwent ASCT. 13 patients relapsed, 1 was refractory and 1 died post-transplant due to Covid sepsis. The third patient, who had relapsed, received R Benda, was found to have multiple myeloma 1 year after therapy, and succumbed to sepsis. 63% were put on Rituximab maintenance.

Conclusions: MCL is a rare, aggressive B cell lymphoma with a lesser incidence in Indian population compared to the world. While aggressive chemotherapy with monoclonal antibody has improved the response rate of patients with nodal MCL, wait and watch strategy remains the backbone of management of leukaemic NNMCL.

Light Chain Deposition Disease: A Rare Plasma Cell Disorder: Case Series

Priyanka Moule, Nitin Gupta, Deepika Gupta, Pallav Gupta

Introduction: Light chain deposition disease (LCDD) is a rare clonal plasma cell disorder characterized by deposition of nonamyloid monoclonal light chains. There is lack of data regarding optimal treatment and outcome of these patients.

Aims & Objectives: We discuss 2 cases of LCDD diagnosed and treated at our centre.

Materials & Methods: **Result**: Case1: A 45 years old male, presented with complaints of generalized weakness, abdominal pain and frothy urine. Investigations revealed Hb-8.8gm/dl, TLC-9.61Thou/dl, plt-160000, serum creatinine-1.46 mg/dL and proteinuria (24 h 1258 mg/dl). Ultrasound showed normal size kidneys. Renal biopsy showed nodular glomerulosclerosis with diffuse staining for kappa light chains along the glomerulus. Serum protein electrophoresis and immunofixation revealed M spike (0.20 gm/dl) of IgG kappa. Serum free light chain assay showed Kappa:Lambda ratio (979/20.3 mg/dl) of 48.227. Bone marrow aspirate revealed 18% plasma cells. Patient was started on Bortezomib-Lenalidomide-Dexamethasone (VRD) protocol. He received 5 cycles and attained very good partial remission. Patient underwent autologous stem cell transplant with melphalan conditioning (140 mg/m2). 12 months post transplant he is on follow up and is on maintenance therapy.

Case 2: 51 year old lady with psoriasis presented with complaints fever and burning micturition. Massive proteinuria (24 h-3397 mg)noted. Ultrasound showed normal size kidneys. Kidney biopsy revealed glomerular disease. IHC highlighted lambda chain nodular deposits in mesangial areas. SPEP & IFE showed M band of lambda light chains. Kappa/lambda ratio was 36.9/1780 mg/dl (0.021). Bone marrow aspiration revealed 19% plasma cells. She was treated with Cyclophosphamide- Bortezomib-dexamethasone (CyBorD) protocol for 2 cycles followed by 3 cycles of VRD. She attained VGPR. She was counselled regarding autologous transplant however patient refused. 3 months later she had a biochemical relapse (increase in Lambda light chains in SFLC). She was then retreated with 4 cycles of VRD. She attained complete remission and underwent autologous stem cell transplant with melphalan conditioning. 15 months post transplant she is under follow up and on maintenance therapy.

Conclusions: Patient survival and tolerance of high-dose chemotherapy appears substantially better in LCDD than systemic AL amyloidosis. LCDD should be aggressively treated with chemotherapy, because achieving a hematologic CR or VGPR prolongs survival, even if advanced renal impairment has supervened.

Case Series of 8 Primary Plasma Cell Leukemia Cases A Single Centre Experience

Deepika Gupta, Priyanka Moule, Jyoti Kotwal, Nitin Gupta

Introduction: Plasma cell leukaemia (PCL) is a rare, aggressive variant of Myeloma characterised by the presence of circulating plasma cells. It is classified as either Primary PCL and secondary PCL.

Aims & Objectives: The primary PCL is very rare and reported to occur in less than 1 per million. The clinical course is aggressive with shorter remissions and survival duration. We report a case series of 8 patients of primary Plasma cell leukaemia.

Materials & Methods: A retrospective review of patients treated in clinical Hematology department of SGRH with Primary Plasma cell Leukemia was performed from 2018 to 2022. In each patient, the clinical and laboratory characteristics were documented at diagnosis. The diagnostic tests utilised includes complete blood counts, Flow-cytometry from peripheral blood sample, bone marrow aspiration and biopsy, FISH testing PET CT whole body/X rays for skeletal imaging, and other samples for biochemistry were done.

The criteria used for diagnosing the patients with PCL is the latest 2021 consensus by IMWG group where Primary PCL is defined by the presence of 5% or more circulating plasma cells in peripheral blood.

Result: 8 patients were diagnosed and treated. The median age at diagnosis was 49 years (34–70) and male to female ratio was 5:3. Most common clinical presentation was bony pains, easy fatiguability and shortness of breath. One patient had skin nodular lesions and breast involvement by plasma cell Leukaemia at relapse and one patient had buccal mucosa plasmacytoma at relapse. Flow cytometry was possible in 6 cases. CD56 was positive in 4 cases and negative in 2 cases,CD19 and CD20 was negative in all the cases and CD28 was variable expressed. FISH was done in 7 and 3 patients were positive for 17p deletion. All the patients were given initially bortezomib/immunomodulator/dexamethasone and 4 patients were consolidated with Auto Peripheral blood stem cell transplant. 6 patients relapsed (median PFS 7 months) and 2 expired and 4 are on 2nd line therapy. 2 patients are in complete remission post auto BMT.

Conclusions: The prognosis of PCL is bad, hence novel agents are preferred followed by transplant. It is recommended that techniques

like Immunophenotyping on peripheral blood should be performed whenever possible.

A Reserve Site Plasmacytoma Occurring in a Extramedullary Site: A Rare Case Report

S.Karthick Velavan, Ashutosh Panigrahi

Introduction: Extramedullary plasmacytomas (EMP) hardly very few cases reported in the literature. Dual occurrence is extremely rare. They seldom occur in the testis and are commonly accompanied by concurrent multiple myeloma at the time of diagnosis. EMP is a rare plasma cell dyscrasia with a better clinical outcome.
Aims & Objectives: CASE REPORT- A 36-year-old gentleman, with no comorbidity, no family history, and a laborer from Odisha attended our department with progressive swelling in the anterior chest wall for the past 3 months, started gradually worsening breathlessness associated with difficulty in swallowing the past 5 days. Advanced with whole-body PET-CT, which showed increased FDG uptake in irregular soft tissue mass in the anterior mediastinum infiltrating chest wall abutting superior vena cava, right atrium, right pericardium, main pulmonary trunk, and increased FDG uptake in enhancing testicular mass lesion involved in left testis associated with hydrocele suggestive of malignancy. Beta HCG and AFP were found to be normal. Testicular swelling was planned for high inguinal orchidectomy, in which tumor cells found to be diffusely immune positive for CD 38 with lambda chain restriction turned out to be testicular plasmacytoma.
Materials & Methods: We started induction chemotherapy with cyclophosphamide, Bortezomib, and thalidomide as per regular doses. EMP is an extremely radiosensitive tumor, control rates in the local area are around 80–100% which is constantly described with moderate doses of radiotherapy. The overall prognosis for patients with testicular plasmacytoma is poor, with high rates of progression to multiple myeloma. Because of the high rates of progression, these patients require close monitoring and long-term surveillance.
Result: The crux of this case report is if any patient with extramedullary plasmacytoma always try to rule out testicular plasmacytoma which is the most common hidden area we try to miss out. The testis is considered a reserve site in multiple myeloma and also in hematological malignancies. Testicular involvement in multiple myeloma from MM usually specifies an aggressive disease with a poor clinical prognosis.
Conclusions: The crux of this case report is if any patient with extramedullary plasmacytoma always tries to rule out testicular plasmacytoma which is the most common hidden area we try to miss out. The testis is considered a reserve site in multiple myeloma and also in hematological malignancies. Testicular involvement in multiple myeloma from MM usually specifies an aggressive disease with a poor clinical prognosis.

Rosai Dorfman Disease-A Rare Case Entitiy Of Eastern India

Sambeetkumarsubudhi, Priyanka Samal,samirsahu Dr sandeepratha

Introduction: Rosai-Dorfman Disease (RDD) is also known as Sinus histiocytosis with massive lymphadenopathy (SHML). It is a rare disorder which is idiopathic in nature.The median age group affected by RDD is ranges from 2 to 79 years. It is characterized by proliferation of white blood cells which are accumulated in the lymph node especially in cervical lymph nodes.
History & clinical case: A 58-year-old male patient, who presented with generalized weakness, head reeling, abdominal distension and occasional constipation for 1 month. On general examination patient was found with bilateral axillary lymphadenopathy.The histopathological findings and radiological reports including CECT chest and abdomen, and PET Scan of the patient excluded all the possibilities of Langerhans cell histiocytosis as well as multicentricCastleman disease and finally diagnosed as Rosai-Dorfman disease.TheMicroscopic Appearance of the cut section shows lymph node with an expanded paracortex and dilated sinusoids. There is a diffuse infiltrate of histiocytes, plasma cells, lymphocytes, prominent emperipolesis was noted. The histocytes express S-100 protein & CD 68. The plasma cell infiltrate is polytypic by in situ hybridization. The residual 'B' zone is highlighted by CD 20. CD 3 marks the interstitial 'T' lymphocytes and CD 138 the plasma cells.The patient was administered with pulse therapy(methylprednisolone 1 g) of steroids followed by oral steroids which was tapered over 6 week. Swollen lymph nodes in the armpits and neck subsided spontaneously after 2 months. The patient was under observation and spontaneous remission occurred after six months.
Unique features: Rosai-Dorfman disease is a rare disorder and-globally there is only 423 cases of RRD reported till today and only 64 cases were reported from India from 1994 to 2022.We report one such case diagnosed as RDD. Almost people of all ages will be affected by this disease but the onset of the disease after the age of 50 is very rare.
Conclusion: RDD can occur alone or related to autoimmune diseases, genetic diseases, and malignancies.The histological characteristics of RDD may be related to Hodgkin's and non-Hodgkin's lymphoma, in which malignant tumours and RDD may occur before or after the same node. For example, RDD-related immune diseases can be seen in up to 10% of cases; systemic lupus erythematosus, idiopathic juvenile arthritis, and autoimmune haemolytic anemia. Once autoimmune diseases, familial causes, and malignancies are ruled out, observation is usually the treatment of choice. Spontaneous remission occurs in 20% to 50% of cases. If the disease is unifocal, surgery can be cured. Immunotherapy, chemotherapy, radiation therapy, corticosteroids, and sirolimus have been used with varying success.

A Rare Case Report of Ocular Adnexal Marginal Zone Lymphoma

Nadeem K, R K Jena, Dibyajyoti Prusty

Introduction: Marginal zone lymphomas (MZLs) are indolent lymphomas arising from B lymphocytes in the marginal zone of a lymphoid follicle. MALT lymphoma is the commonest MZL and stomach is the commonest site for MALT lymphoma. Other involved sites include ocular adnexa, salivary glands, lung, thyroid etc., but the nonspecificity of the clinical presentation and lack of wide availability of specific IHC markers makes the diagnosis of such MALT lymphomas a challenging one. Here we present the case report of such a rare case of Ocular Adnexal Marginal Zone Lymphoma (OAMZL).
Aims & Objectives: To evaluate, diagnose and treat a suspected neoplastic swelling (?OAMZL) of the orbit.
Materials & Methods: A 60 year old man presented with a slowly progressing right orbital swelling with deviation of eye-ball along with epiphora in 2017.
CT head revealed a right orbital mass in superomedial aspect, with both intra- and extraconal compartments with associated invasion of extraocular muscles, optic nerve and erosion of orbit; likely malignant.

PET Scan show a metabolically active soft tissue lesion involving right orbit with intracranial extension.

Biopsy: diffuse interfollicular proliferation of small-medium sized cells with moderately dispersed chromatin and a few plasma cells. On IHC, these cells were Positive for CD19 and CD20, and Negative for CD3, CD5, CD10, CD23, and Cyclin D1.

Result: Diagnosed as ocular adnexal marginal zone lymphoma stage IE and treated with clarithromycin 500 mg for 6 months. Patient was lost to follow up and again presented with increased swelling after 4 years. His revised evaluation put him in stage IIAE, MALT IPI-I. He was put on BR Chemotherapy. After 4th cycle of BR, the swelling is completely vanished clinically and repeat radiology revealed a reduction in tumour size from 3.8 × 3.2 × 2.9 cm to 1.9 × 1.8 cm.
Conclusions: OAMZL is a challenging entity both diagnostically and therapeutically as there are no definite guidelines exist so far. But a systematic diagnostic approach and implementation of therapeutic approaches from existing literature could result in improved outcome as shown in this case report.

Is Bone Marrow Obsolete in Newly Diagnosed ITP-A Dilemma-A Rare Case of Multiple Myeloma Presenting as ITP in a Known and Treated Case of Ca Breast

Parshav Jain, Priyanka Samal, Samir Sahu, Harsh Bardhan

Introduction: Immune thrombocytopenic Purpura is an acquired disorder in which there is immune-mediated destruction of platelets and possibly inhibition of platelet release from the megakaryocytes. The association of multiple myeloma with ITP is rare and only a few cases are reported.
Case Report: A 51-year-old female patient presented with petechiae and purpura over body since one and half months. She was a diagnosed with Carcinoma Breast in March 2018, and underwent Modified Radical Mastectomy in June 2018 along with Chemo and Radiotherapy in July 2018. On examination there was wet purpura in oral cavity and her CBC showed- Hb-13.4 gm%, TLC- 6,500/mm^3, platelet count- 10,000/mm^3 without any atypical cells in blood smear. Bone Marrow examination was done for evaluation for thrombocytopenia. The aspiration was reported as hypercellular marrow with Plasmacytosis [15% plasma cells].Immunophenotyping of marrow aspirate was suggestive of clonal plasma cells with lambda light chain restriction. Her serum electrophoresis and Immunofixation diagnosed it as IgA lambda Multiple myeloma. PET scan was done which revealed lytic lesion in the skeleton and bone marrow infiltrative disease while the breast and other organs had no abnormal FDG uptake. The patient was managed with steroids as front line therapy for ITP and achieved a complete response. After one and half months, she was initiated on antimyeloma therapy. Post 4 cycles of chemotherapy, she underwent autologous stem cell transplantation, following which she continues in complete remission for both her malignancies.
Discussion: In this case, we emphasize the fact that, even if a peripheral blood smear and CBC parameters favour ITP, a malignancy may be missed if a bone marrow is not done to exclude other causes of thrombocytopenia.
Conclusions: The pathogenesis of ITP in MM has been hypothesised to the immune alterations promoting the generation of autoimmune platelet antibodies by the malignant plasma cells.

Non Hodgkin Lymphoma: An Audit of Histology, Clinical Features and Outcomes of Treatment in Patients Treated from 2011–2018

Pranita Mishra, Reena Nair,Vivek Radhakrishnan, Mammen Chandy, Arijit Nag, Saurabh Bhave, Jeevan Kumar, Mayur Parihar, Deepak Kumar Mishra, Lateef Zameer, Indu P, Sushant Vinarkar,Serya Das, Bivas Chakraborty, Amrita Paul, Susmita Dasgupta

Introduction: Many papers describe the histological subtypes of non Hodgkin Lymphoma (NHL) in literature from India but systemic reports of descriptive epidemiology and treatment outcomes from India are scarce.
Aims & Objectives: This study aims to survey the clinical spectrum of NHL in terms of epidemiology, pathological subtypes, stage, prognostic factors and outcomes of first line therapy.
Materials & Methods: A tertiary cancer center in Eastern India audited the histology, clinical features and survival of adult (≥ 18 years) NHL treatment naïve patients. Using the Hospital Management System (HMS), data was collected from 2011 to 2018 for all treatment NHL patients. The diagnostic pathology, according to the WHO classification 2008, clinical features and survival of 1432 patients was loaded on the OncoCollect software and analysed. Standard first line treatment consisted of CHOP regimen with or without etoposide for NKTCL, Rituximab for B-cell lymphoma. Radiotherapy was reserved for bulky disease at presentation. All patients were followed up till June 2022.
Result: The median age of patients was 58 years (range 18–90 years). Male to female ratio was 2.2:1. Of the total 1432 patients, B-cell lymphoma comprised 91% and T/NK cell lymphoma (TNKCL) comprised 9%. The leading histopathological subtypes were 54% diffuse large B-cell lymphoma (DLBCL), 10% follicular lymphoma (FL), 13% small lymphocytic lymphoma (SLL)/chronic lymphatic leukemia (CLL) and 6% marginal zone lymphoma (MZL).
Extranodal presentation was seen in 32% and was common in DLBCL lymphomas. Early stage disease was present in 37% and advanced stage disease in 63%.

The response to 1st line therapy was 82% (CR + PR) for DLBCL, 86% for FL, 65% for CLL/SLL, and 65% for TNKCL. With a median Follow Up of 33 months, the 5-year progression free survival (PFS) was significantly longer for B-cell lymphoma than NKTCL (65% versus 32% respectively, $p < 0 >$
Conclusions: The epidemiological, clinical spectrum and outcomes of NHL are observed to have some differences from the Western countries as well as the far east.

Signature Of Nodal Castleman Disease in Bone Marrow Trephine Biopsy

K Vamsi Krishna, Somanath Padhi, Satarupa Mohapatra, Pritinanda Mishra, Ashutosh Panigrahi

Introduction: Castleman disease (CD) is a rare non clonal lymphoproliferative disorder that includes both unicentric (UCD) and multicentric (MCD) subtypes. While UCD presents like a mass like lesion at organ specific anatomic sites (both nodal and extra nodal), clinical presentation of MCD variant may range from significant B symptoms mimicking a lymphoproliferative neoplasm (LPN) or this may be a part of POEMS syndrome. The bone marrow (BM) involvement in CD is sporadically described in the literature.
Aims & Objectives: To describe the clinicopathological and BM histomorphological changes in a case of nodal CD with review of published literature (2010–2021).
Materials & Methods: A middle-aged HIV seronegative male presented with significant B symptoms in the form of weight loss, ascites, bilateral limb edema, and lymphadenopathy (cervical, axillary) suspected to be of lymphoma. The cervical and axillary lymph node (LN) biopsy was suggestive of hyaline vascular type CD. He subsequently underwent BM biopsy (BMBx) to rule out POEMS syndrome or a lymphoma. We present the data on BM changes in 80 cases of nodal CD published in the literature.
Result: The BMBx in our case showed hypercellularity with adequate trilineage hematopoiesis (TLH), increased megakaryocytes and presence of multiple reactive interstitial lymphoid aggregates encircled by increased number of reactive plasma cells. Besides these, there was perifollicular increased vascularity along with presence of

penetrating vessels which gave a 'lolly pop' like appearance similar to that seen in LN biopsy. Immunohistochemical staining for Human Herpes Virus 8 (HHV8) was positive from LN, but negative from the BM. His POEMS related work up was negative. Review of BM morphology in 80 cases of CD (47 HIV +) revealed a normo to hypercellular marrow with adequate TLH in all, benign lymphoid aggregates in 25 (31.25%), plasmacytosis in 74 (92.5%) [monoclonal in 6/80 (7.5%) as a part of POEMS syndrome], and increased perifollicular vascularity in some. HHV8 association was noted among 12/65 (18.5%) where this was tested. Work up for lymphoma, pyrexia of unknown origin, cytopenia (s), and POEMS syndrome were the common indications for performing BM evaluation in these cases.
Conclusions: BM involvement in CD are rarely reported in literature. Lymphoid aggregates, plasmacytosis, and increased perifollicular vascularity should be looked for possible marrow involvement and be investigated for possible HHV8 association.

Autoimmune Hemaolytic Anemia with Mantle Cell Leukemia and Response to Btkinhibitor

Sohomghosh, Priyanka Samal Samir Sahu

Introduction: AIHA has been associated with various types of lymphomas but very rarely with Mantle cell lymphoma. It is more often associated with the indolent subtype of MCL with a leukemic presentation and often necessitates the treatment of the lymphoma rather than just managing with steroids as antihemolytic strategies.
Clinical case: A 67 year old female patient presented with the complaint of fatigue for requiring 9 units of blood transfusion in last one and half months with mild icterus. Her baseline tests revealed-Hb- 5.8 g/dL, MCV 104 fl, TLC- 25.65 $\times$ 10^9/L,N16,L72, platelet count—1.75 $\times$ 10^9/L, corrected reticulocyte 8.39%, LDH 455 U/L, total/direct bilirubin 2.91/0.7 mg/dL, Direct Coombs Test (IgG and Cd3) 4 + . anti-nuclear antibody (ANA) negative negative. Peripheral smear showed marked agglutination, spherocytes and polychromasia with atypical lymphocytosis. Bone marrow biopsy done confirmed the lymphoma infiltration of marrow and hence considered stage IV disease. IHC was positive for focal cyclin D1 and flow cytometry finding were CD5 positive, CD10 & CD23 negative), favours a possibility of pleomorphic/blast variant of mantle cell lymphoma involving marrow.With AIHA diagnosis, the patient received 4 unit PRBC,1 mg/kg/day methylprednisolone and also treated with RITUXIMAB and bendamustine 6 cycles as definitive therapy with maintenance for 2 years. Unfortunately she relapsed 2 months after her 12^{th} dose of Rituximab maintenance again with warm type AIHA. This time Flow cytometry of the lymphoid cells was negative for CD 20. She was started on 1 gm Methylprednisolone but had no improvement in Hb even after 14 days of steroids @ 1 mg/kg. Hence, she was started on Ibrutinib to which she responded well and now continues be in remission while on therapy.
Conclusion: Elderly patients presenting with AIHA must be evaluated to rule out underlying malignancies such as lymphoproliferative disorders for early diagnosis and proper management. Post exposure to anti CD20, Rituximab, there may be downregulation of CD 20 on the tumor cells, which necessitates the use of Bruton kinase inhibitors for an adequate response.

A Rare Incidence of Multiple Myeloma Presenting as Pleural Effusion

Vishnupriya Duddugunta, Revanth Boddu, Kundan Mishra, Sandeep Goyal, Nidhi Yadav, Suman Pramanik

Introduction: Multiple myeloma (MM) is a plasma cell neoplasm, predominantly involving the bone marrow and skeletal system although extramedullary tissues may be infiltrated as well. The reported incidence of pleural effusion in patients with MM is about 6%, and malignant pleural effusion occurs only in < 1 >
Aims & Objectives: In this case report, we present a very rare and unusual presentation of multiple myeloma as malignant pleural effusion, treated successfully.
Materials & Methods: A 55-year-old man presented with complaints of progressive breathlessness, and 4 kg weight loss over 2 months. On examination, he had pallor and tachypnea. Auscultation revealed decreased breath sounds in the left infra-scapular and infra-axillary areas. Chest X-ray showed left-sided pleural effusion. Computed tomography showed lobulated pleural and fissural effusion, with collapse consolidation of the underlying lung on the left side. In view of loculated effusion, pleurocenetesis was done and a pigtail was inserted. The pleural fluid was yellowish in color and biochemistry was suggestive of exudative effusion. Cytology showed 800 WBC/μL with bilobed and multilobed plasma cells. On flow cytometry, 58% of all nucleated cells were CD38, CD138, CD56, and CD200 positive with kappa light chain restriction. After a thorough evaluation, a final diagnosis of myelomatous pleural effusion withIgG kappa type of multiple myeloma was made. He was restarted on bortezomib, lenalidomide, and dexamethasone regimen with radiotherapy to the skeletal lesions. Within two weeks, he had relief from pain and dyspnea. Four weeks later, repeat imaging showed clearing of pleural effusion and complete relief from dyspnea.
Result: The incidence of myelomatous pleural effusion (MPE) is reported to be < 1 >
Conclusions: Though MPE is rare, its presence indicates the aggressive nature of underlying myeloma and guides towards early initiation of treatment.

Rare Case Reports of Multiple Myeloma Masquerading as Acute Kidney Failure

Noopur S. Patil, Vrinda K. Kulkarni, Meenakshi Balasubramanian, Sarang Waghmare, Nisha Gyanchandani, Sameer Agrawal, Rashmita Nevgi, Avinash Chalke, Dashrath Sawant

Introduction: Multiple myeloma (MM) is a neoplastic plasma cell dyscrasia identified by anemia, recurrent infections, increased serum and/or monoclonal protein in urine, osteolytic bone lesions, hypercalcemia and renal failure. The ongoing renal failure in MM results from tubular nephropathy because of circulating paraproteins secreted by plasma cell clones, most commonly immunoglobulins and free light chains leading to cast nephropathy. The incidence of cast nephropathy is 30% in MM.
Aims & Objectives: Our aim in the paper, is to report that advanced age, concomitant chronic renal failure with unknown cause and anemia should always bring MM to mind. In such cases SPE with urine & serum Immunofixation along with Kidney biopsy must be mandated to rule out the exact cause of Acute kidney Injury.
Materials & Methods: We report three cases following up in Nephrology OPD since 5-6 months with chronic kidney disease requiring MHD three times a week. In view of non-resolving anemia even after correcting the iron deficiency and starting on an EPO Derivative, further investigations like Serum electrophoresis with free light chain and ratio were advised.
Case 1: 50/F presented with AKI on CKD, BMA showed 22% plasma cells.
M Band present, SFLc Raised with Abnormal Ratio.
Case 2: 55/M presented with CKD Stage VD, BMA showed 30% plasma cells, Congo Red for Abdominal fat pad biopsy showed Apple green birefringence.
Discrete M band, SFLc Slightly raised with Abnormal Ratio.
Case 3: 35/M presented with RPGN; BMA showed 42% plasma cells.

Absent M Band, with Highly raised SFLc with Abnormally High Ratio.

In advanced examination B2-Microglobulin was increased, Urine BJ protein Positive, Serum Calcium > 11 mg/dl and Serum Creatinine > 5 mg/dl.

Finally, to know the cause of kidney disease, a kidney biopsy was done, Histology showed Fractured tubules & Immunofluorescence showed Light chain restriction within the casts.

Result: The cases were labelled as MM with Cast nephropathy (light chain disease) and VRD (Bortezomib, Lenalidomide, Dexamethasone) regimen was given. After completion of 1st cycle patient improved symptomatically with reduction in Free light chain assay, Correction of Anemia, with Reduction in requirement of MHD and improvement in quality of life.

Conclusions: The presence of elevated FLCs and a perturbed sFLC κ:λ ratio is a key marker for detecting plasma cell malignancies and has been shown to be an important indicator of myeloma in the presence of AKI.

In patients presenting with an AKI where the underlying pathology is unknown, myeloma should be investigated as a potential cause; this requires a robust screening method to be in place.

In summary, Cast nephropathy with MM are an area in which early diagnosis is necessary, so that a thorough evaluation of renal function followed by a renal biopsy is always recommended as a standard approach to diagnosis and early treatment.

Melphalan-Associated Encephalopathy Following Autologous Stem Cell Transplant: A Transplanter's Nightmare!

Suchita Shinde, Guntiboina Vinay Anand, Akshay Lahoti, Arjin Philips Jacoby, Mita Roy Chowdhury, Pralay Shankar Ghosh, Sudipta Mukherjee, Shantanu Bagchi, Saurabh Jayant Bhave, Jeevan Kumar Garg, Reena Nair, Arijit Nag

Introduction: High dose Melphalan with autologous stem cell recue (HDT-ASCT) is an effective consolidation strategy in patients with multiple myeloma (MM), even for those with pre-existing renal disease, and favourably impacts disease outcomes (1). Neurotoxicity with melphalan is rare but serious complication of melphalan (2) that can be managed with adequate supportive care.

Aims & Objectives: –

Materials & Methods: –

Result: Case report: A 63-year-old gentleman with type II diabetes mellitus, hypertension, and CKD, diagnosed to have IgG Kappa MM with ISS-III and standard risk cytogenetics (mSMART) underwent HDT-ASCT in a stringent complete response (sCR). A reduced intensity conditioning of Melphalan 140 mg/sq.m was planned for this patient considering his baseline renal dysfunction (CrCl: 16 ml/min). He developed grade 3–4 mucositis on D + 4 and had febrile neutropenia on D + 5 which was managed as per standard institutional practice. This was followed by worsening mentation by D + 8 and worsening oliguria, necessitating haemodialysis (HD). His neurological status worsened (GCS: E2V2M2: 6/15) without any focal neurological signs or deficits. EEG showed a global slowing. MRI and CSF studies (including viral and BioFire panel and bacterial/fungal/mycobacterial studies) were non-contributory. Considering the timeline of events, lack of response to HD and infection control measures, the possibility of melphalan-induced neurotoxicity was considered. With continued supportive care, the patient's sensorium started to improve with complete recovery by D + 24. Renal functions started improving subsequently and he was discharged on D + 30.

Discussion: Melphalan associated neurotoxicity has been reported in 1–2.

Conclusions: Melphalan-induced encephalopathy is a rare complication following HDT-ASCT. If suspected, it is important to provide effective critical care with close observation of the patient's neurological condition as this is known to be associated with complete neurological recovery.

A Rare Case of Splenic Marzinal Zone Lymphoma in a Teenage Girl

Keerthyvarman M, S.M.Sujatha, Sivaraman, Naveen Kumar

Introduction: Pancytopenia is a condition in which there is a lower than normal number of red cells,white blood cells and platelets in the blood. Pancytopenia occurs when there is a problem with blood forming stem cells in bone marrow. Signs and symptoms include fatigue,weakness,dizziness,trouble breathing, tachycardia,fever,paleskin,paleskin,purple or red spots on the skin,rash,easy bruising and abnormal bleeding. Pancytopenia may be caused by certain autoimmune,bone marrow or genetic disorders. It may also be caused by infection,poornutrition,pregnancy,cancer treatment or exposure to toxin,chemicals or drugs.

Aims & Objectives: A young teenage girl with PUO,pancytopenia and splenomegaly it is very important to rule out hematological malignancies (lymphoma/leukemia)).

Materials & Methods: This is a case report based on case seen in Stanley medical college and hospital under department of general medicine. Laboratory investigations and procedures are done in pathological,biochemistry and microbiology laboratory in Stanley medical college and hospital. Imaging is done by Radiology department of Stanley medical college and hospital.

Result: A 18 years old female old history of pulmonary tuberculosis treatment 3 years back,H/o previous admission for Anaemia and thrombocytopenia is present 7 years back,H/o bilateral chronic otitis media present. Now she is admitted history of easy fatiguability since 5 years increase since 2 weeks, H/o recurrent fever with cough and cold present for past 1 year, H/o shortness of breath increased on exertion since past 1 month,H/o headache present for past 2 weeks. On examination she was pale, no significant lymphadenopathy was seen and per abdomen splenomegaly was seen.Peripheral smear showed RBC—microcytic hypochromic anaemia, WBC- decreased in number N-24%,L-21% with reactive lymphocytes, Platelets-decreased in number. Final impression of peripheral smears revealed a microcytic hypochromic anaemia,leukopenia with relative lymphocyte predominance, thrombocytopenia. Ultrasound abdomen revealed splenomegaly (17.5 cm).CT scan of brain normal,CT of thorax- no significant abnormalities,CT Of abdomen also revealed splenomegaly and otherwise normal. ANA profile negative, karyotyping -normal,Direct coombs test negative, viral markers negative.We proceeded with bone marrow biopsy after improving hemoglobin and platelets count.Bone marrow biopsy revealed Trilineage hematopoeisis with possiblity of lymphoproliferative disorder.

Conclusions: This is a very uncommon presentation of a lymphoproliferative disorder in a teenage girl with splenomegaly alone.Pancytopenia is a scenario with diagnostic challenge. The range of potential causes is bewildering,signs and symptoms overlap substantially,and many diseases presenting with pancytopenia are life-threatening if not recognized and managed properly. Therefore

clinicians must be familiar with clinical scenarios that should prompt evaluation of blood counts and hematology referral.

Treatment Experience of NK T Cell Lymphoma from a Tertiary Care Hospital in Eastern India

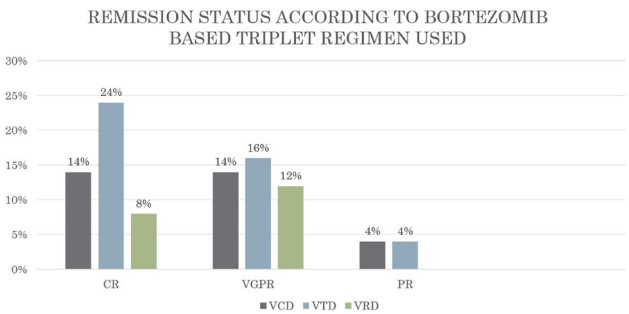
Sayan Ghoshal, Jeevan Kumar

Introduction: Extra nodal natural killer (NK)/T-cell lymphoma is an aggressive malignancy of putative NK-cell origin, with a minority deriving from the T-cell lineage. Pathologically, the malignancy occurs in two forms, extra nodal, nasal type; and aggressive type. Lymphoma occur most commonly (80%) in the nose and upper aerodigestive tract, less commonly (20%) in non-nasal areas (skin, GI tract, testis, salivary gland) and rarely as disseminated disease with a leukemic phase.
Aims & Objectives: to evaluate the clinical presentation, lab findings, spectrum of treatment modalities of NK T cell lymphoma and to assess the efficacy of chemotherapy used for treatment.
Materials & Methods: A retrospective study was conducted from November 2011 to June 2022 in the Department of Clinical Hematology and BMT, TMC, Kolkata. A total of 18 patients were included in the study. All patients were assessed by nationality, clinical presentation, lab parameters, lines of treatment, complications and response assessment.
Result: Out of 18 patients, 9 (50%) were females, 9 (50%) patients were from Bhutan with most incidence of NK T cell lymphomas occur in the age group of 35–45 years. Nasal blockage was the most common clinical presentation. Four (22%) patients presented with anemia, where as in one patient (5%) bone marrow was involved and two patients (11%) presented with lung nodule. SMILE chemotherapy was given upfront to eight patients (44%) and in one patient it was given as second line post CHOP chemotherapy. End of treatment PET scan showed complete metabolic response in six patients (33%). Febrile neutropenia and cytopenia were the most common complication post SMILE chemotherapy followed by sepsis. Five patients (27%) died during treatment, out of which three died of neutropenic sepsis. Nine patients (50%) lost follow up during the course of treatment, so essentially four patients (22%) were alive till date.
Conclusions: In stage I/II diseases, combined chemotherapy and radiotherapy (sequentially or concurrently) is the best approach. Conventional anthracycline-containing regimens are ineffective and should be replaced by SMILE protocol, preferably including L-asparaginase. Prognostic models taking into account presentation, interim and end-of-treatment response assessments are useful in triaging patients to different treatment strategies.

Initial Treatment Outcome of Newly Diagnosed Myeloma Patients in Tertiary Care Private Hospital in Eastern India

Aeshrat Bano, Anupam Chakrapani, Debmalya Bhattacharya, Soumya Bhattacharya

Introduction: Multiple myeloma is characterized by significant heterogeneities in clinical manifestations and prognosis. The International staging system represents today one of the most widely used staging system for patients with MM. Several new therapeutic options introduced for the treatment of MM during the past 2 decades include immune-modulatory drugs(iMiD) eg. Thalidomide and proteasome inhibitors (PI) eg.Bortezomib. Currently, 3-drug combinations including an iMID, a PI and a steroid are commonly used for remission induction prior to ASCT. But there are some variations in chosing the initial regimen from centre to centre.
Aims & Objectives: 1. To assess remission status according to the ISS staging and regimen used (VCD/VRD/VTD). 2. To assess impact of bortezomib based triplet regimen on autologous stem cell transplant
Materials & Methods: A single institution retrospective study constituting 100 consecutive newly diagnosed multiple myeloma patients visiting the OPD of Clinical hematology department of Apollo multispeciality Hospitals from June 2020 to June 2022 were taken into consideration. Demographic data, blood parameters including b2 microglobulin and albumin were noted. 50 consecutive patients who were administered at least 4 cycles of bortezomib based triplet regimen were included in the current analysis. Patients belonging to all ages and both genders were included.
Result: 42%,38% and 20% of patients were administered VTD, VCD and VRDrespectively. 30%,30% and 40% of patients belonged to ISS I,ISS II and ISS III respectively. The CR, VGPR and PR rate of the studied population was 46%, 42% and 8% respectively. Patients in ISS I category were more likely to achieve CR status (73% vs 46% in ISS II and 25% in ISS III).
42% of patients underwent successful autologous stem cell transplant after induction regimen.
Conclusions: Bortezomib based regimens showed high response rate (46% CR, 42% VGPR). Patients belonging to ISS I had better chance of remission, irrespective of the triplet regimen used. All patients who underwent autoBMT successfully engrafted, thereby suggesting that Bortezomib based regimens do not impact the quality of stem cells. More detailed analysis after completion of the full analysis with 100 patients will throw more light upon the possible significantly related parameters.



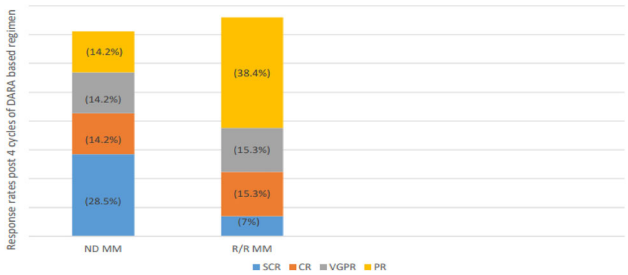
Daratumumab in Multiple Myeloma Patients: A Real World Single Center Experience from North East India

Sonal Paul, Jina Bhattacharyya, Smita Das, Damodar Das, Riju Rani Deka, Sewali Deka Talukdar, Dhanjit Haloi, Biswaprakash Patri

Introduction: Daratumumab is an IgG1 kappa monoclonal antibody against CD38, overexpressed by myeloma cells. It acts by various mechanisms namely, complement mediated cytotoxicity, antibody dependent cell mediated cytotoxicity, antibody dependent cellular phagocytosis, and apoptosis. It has been FDA approved for newly diagnosed multiple myeloma (NDMM) as well as for relapsed refractory (R/R MM) cases. Herein, we describe a single institution experience of the efficacy of DARA based treatment in NDMM and R/R cases.
Aims & Objectives: (1) To study the efficacy of Daratumumab in Newly diagnosed and relapsed refractory multiple myeloma patients in our population.
(2) To study the tolerability and adverse effects of Daratumumab.
Materials & Methods: Patients treated at the Dept of Clinical Haematology, Gauhati Medical college and hospital with Daratumumab between February 2021 to June 2022 were included. Data was collected by review of outpatient records and analysed for patient demographics, disease characteristics, treatment and outcomes.
Result: 20 patients (7 NDMM and 13 R/R MM) were included, with a median age of 58 years (range 44–81).Dara was given along with

bortezomib, cyclophosphamide and dexamethasone(VCD) in 6 of the newly diagnosed patients while one received it with VR(lenalidomide)D.The 13 RRMM patients had received a median number of 3 prior therapies with all patients being previously exposed to PI and IMID.2 patients relapsed post autologous transplant and were treated with Dara-Rd and Dara- K(Carfilzomib)d respectively. Rest of the RR patients received a quadruplet regimen(Dara-VCD/VRd). All NDMM patients presented with high risk characteristics.Post 4 cycles, the overall response rate was 71% in the NDMM, 76% in RRMM patients with 28.5% (2/7) achieving sCR(NDMM) and 7%(1/13) achieving sCR(RRMM). 5 patients, 2 newly diagnosed and 3 RRMM expired due to presence of multiple comorbidities and probably due to advanced stage of presentation of disease. Overall, DARA based regimens were well tolerated with no adverse events.

Conclusions: Present real life experience reveals that Dara based regimens have greater efficacy with acceptable tolerability in newly diagnosed MM and in advanced patients with RRMM, as supported by most clinical trials.



Plasmablastic Lymphoma: A Case Series of 7 Cases from a Tertiary Care Oncology Institute in India

Abhishek Kumar, Reena Nair, Saurabh bhave, Jeevan kumar, Arijit Nag, Deepak Mishra

Introduction: Plasmablastic lymphoma (PBL), initially described in the oral cavity of HIV positive patients, is now recognized as a distinct aggressive and rare entity of diffuse large B-cell lymphoma by the World Health Organization classification. Very few cases have been reported as single case reports and small case series and there is paucity of data regarding the clinico-hematological presentation and treatment outcomes.

Aims & Objectives: To analyze the clinico-hematological presentation, sites of involvement, serology status, Immunophenotype, proliferation index and treatment outcomes in cases of Plasmablastic Lymphoma.

Materials & Methods: We retrospectively analysed total 7 cases of Plasmablastic Lymphoma in our institute during the last 10 years, and all the relevant data were retrieved from our Electronic medical records.

Result: Median age of presentation was 60 yrs, 2 patients had nodal plus extranodal involvement, and 2 pts had only nodal involvement. Three pts presented only with extra-nodal involvement. Four pts presented with stage II/IV disease (57%). Common extranodal sites were PNS in 2 pts, Pancreas in 2 pts, and other sites were liver, ovary, breast, bone and kidney. All 7 pts had CD 20 negative and CD 138 positive immunophenotype. EBER ISH was done in 3 pts and it was positive in all 3 pts. Median Ki 67 Index was 85%. Five out of 7 pts were treated with EPOCH and 2 with CHOP chemotherpy as 1st line regimen, all 7 patients went into CMR. Two of those were consolidated with Auto HSCT, out of which 1 had relapse, so underwent Allo-HSCT and patient retained remission status till date now. Median Five year OS was found to be 69%. Out of total 7 pts, 4 pts had a relapse and the median PFS was 18 months.

Conclusions: Our study shows that Plasmablastic lymphoma has a heterogenous clinical presentation and can have nodal/extra nodal involvement, early or late stages at presentation. It has been shown to be associated with poor response to therapy, but our study and few other case series have showed improved outcomes with chemotherapy and/or ASCT. Majority of cases, thought to be associated with HIV positive status, but majority cases in our study were seronegative. So more case series will give us a better understanding of disease behaviour and treatment outcome.

Results Of Interim PET-Guided Therapy in Pediatric Hodgkin's Lymphoma: A Single-Center Experience

Jhasaketan Nayak, Sashikant Singh, Karthik Kumar, Jasmine Porwal, Gaurav Dhingra, Uttam Kumar Nath

Introduction: Hodgkin's lymphoma (HL) is one of the common lymphomas in children. Currently, there is no uniform risk stratification for paediatric HL. Assessment of response to chemotherapy by interim positron-emission tomography (PET)-CT identifies patients who may benefit from therapy escalation.

Aims & Objectives: To analyse the outcomes of interim PET-CT guided treatment in paediatric Hodgkin's lymphoma patients in a tertiary hospital.

Materials & Methods: Our prospective study enrolled total 30 CHL patients of age $\leq$ 18 years, between April 2018 & February 2022. Baseline staging PET-CT scan was done for all patients. Advanced stage disease was defined as stages III & IV. The patients were treated with either ABVD or escalated BEACOPP as first line chemotherapy. Interim PET-CT with Deauville score was done after 2 cycles of chemotherapy. Patients with Deauville scores $\leq$ 3 received 2–4 cycles of ABVD depending on baseline disease stage. Patients with Deauville scores 4–5 on interim PET-CT received 4 cycles of Escalated BEACOPP chemotherapy if deemed fit, followed by end-of-treatment PET-CT for response assessment. Patients received radiation therapy as per standard indications.

Result: Median age of patients was 11 years (range 3–18 years). Majority (80%) had advanced stage disease and 20% had bulky disease. Interim PET-CT could be done in 28 patients, out of which 23 patients (82%) had Deauville score $\leq$ 3. Therapy was intensified to escalated BEACOPP regimen in 5 patients with Deauville score $\geq$ 4 on interim PET-CT. Two patients defaulted treatment after two cycles ABVD; one of them died due to unknown cause, and the other was lost to follow up. Overall, 26/28 patients (93%) achieved complete response (CR) with first-line treatment. One patient with primary refractory disease died of early relapse post-autologous transplantation. One child who achieved CR with 6 cycles of ABVD relapsed 17 months after completion of treatment. Interim PET-CT & end-of-treatment results are summarized in Table 1.

Conclusions: Our single-centre experience suggests that interim PET-CT should be incorporated in treatment algorithm to guide subsequent therapy, with the goal of minimizing long-term treatment-related toxicity & is valuable predictor of end-of-treatment response in paediatric Hodgkin lymphoma (Fig. 1).

Precursor B Cell Lymphoblastic Lymphoma Presenting Rare Case Report

Santosh kumar, Avinash Kr.Singh, Divyakrishna, Ankit Kumar, Narmata Sinha, Khursid Mallick

Introduction: Lymphoblastic lymphoma (LBL) is a rare subtype of non-Hodgkin's lymphoma, more common in children than in adults. This case describes the rare presentation of precursor B cell lymphoma presenting as backache.

Table 1 Response on Interim PET-CT& End of Treatment

CHL disease stage	Interim PET-CT after 2 cycles [ABVD = 29; Escalated BEACOPP = 1]			Subsequent chemotherapy		End of treatment response
	Deauville 1–3	Deauville 4–5	Not done	ABVD × 2–4 cycles	Escalated BEACOPP × 4 cycles	
Early stage [n = 7]	6	1	0	6	1	CR = 6 Refractory = 1
Advanced stage [n = 23]	17	4	2	17	4	CR = 20 Refractory = 1 Defaulter = 2

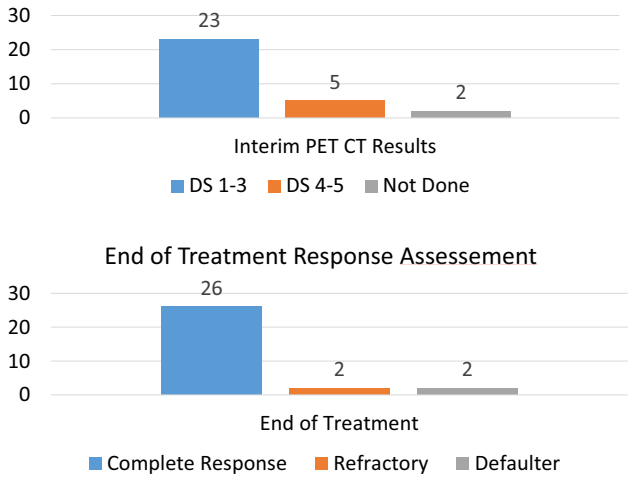


Fig. 1 Response on Interim PET-CT& End of Treatment

Aims & Objectives: To notify rare cases.
Materials & Methods: Actual investigated cases.
Result: A 39 Year old male patients, presented to us with c/o of backache since 10 days, he was evaluated for same, routine investigations BMA/BX and pet CT were done and bone marrow biopsy s/o necrotic marrow, PET CT-SHOWED metabolic active lesion over rt ala of ischium according to above investigations there was no conclusive diagnosis(we have r/o Multiple myeloma and acute leukaemia and lymphoma).Biopsy done from metabolic active ala of rt ischium also s/o nectrotic marrow, IHC were sent –reported necrotic marrow comments not possible, re-biopsy done,IHC Revealed B-Lymphoblastic lymphoma. Details of case we will discuss during oral presentations.
Conclusions: This case highlights the need to be vigilant to unusual presentations of lymphoma in children and adults. It shows the rarity of precursor B cell lymphoblastic lymphoma (LBL) and its morphological similarities to acute lymphoblastic leukaemia.

Incidence, Clinical Spectrum and Reversibility of Renal Dysfunction in Newly Diagnosed Multiple Myeloma

Priyanka Panigrahi, Sudha Sethy, Sarat Chandra Singh, Rabindra Kumar Jena

Introduction: Multiple myeloma is a clonal plasma cell neoplastic disorder with renal involvement being the most common complication of disease affecting the prognosis, treatment and survival of the patient. 20–40% have baseline renal involvement with 1–13.
Aims & Objectives: (1) To estimate the incidence and clinical spectrum of renal dysfunction in newly diagnosed (ND) Multiple Myeloma.
(2) To assess the reversibility of renal dysfunction and its related factors post chemotherapy in our institution.
Materials & Methods: ND-MM diagnosed by International Myeloma Working Group Revised Diagnostic Criteria.
Renal dysfunction defined by Sr. Creatinine $\geq$ 2 mg/dl at diagnosis and/or estimated GFR < 50 ml >
Exclusion of diabetic, Hypertensive, MGUS, smoldering myeloma, solitary plasmacytoma, non-secretory myeloma and light chain disease.
Treatment—BTD with 1.3 mg/m^2 bortezomib, 200 mg thalidomide, 40 mg once a week Dexamethasone repeated for 4–6 cycles.
Reversal of renal function defined as a Sr. Creatinine $\leq$ 1.5 mg/dl assessed after every cycle.
Those who achieved good response (> VGPR), < 65 >
Statistical analysis was done by SPSS version 25
Result: Total no. of patients screened—98
Total no. of patients with renal impairment—21
No. of male patients—14(66.6%)
No. of female patients—7(33.3%)
Mean duration of illness before chemotherapy—6 months (1mo-1 yr)
Complete Reversal of renal function—18(86%)
Myeloma subtype
IgG- kappa—14(66.6%)
IgG-lambda—4 (19%)
IgA—0
Renal amyloid—3 (14%)
Stage of myeloma
Stage 2B—5 (23.8%)
Stage 3B—16 (76.2%)
Conclusions: In our study, Incidence of renal dysfunction in ND-MM is 21.4%
Majority of patients were in an age group of 40–55 years.
Reduced duration of illness before CT had better outcome.
Reversal of renal function achieved in 86% patients.
Non-amyloidosis renal disease and early initiation of treatment had significant reversibility of renal dysfunction.
Monoclonal light chain deposition commonest cause of renal impairment.
Lambda light chain (19%) had bad outcome.

Clonal Evolution of Multiple Myeloma

Gurvinder Kaur, Lingaraja Jena, Ritu Gupta, Akanksha Farswan, Anubha Gupta,Sriram K

Introduction: Somatic mutations in Multiple Myeloma (MM) tend to evolve in sync with progression and regulate response to therapy. However, there are limited studies on patterns of clonal evolution and their interactions in MM.
Aims & Objectives: The aim of this study was to compare mutational landscapes at diagnosis and on progression of MM and identify clonal mutations.
Materials & Methods: Whole exome datasets for 76 patients at multiple time points and enrolled under the MMRF CoMMpass study were assessed for clonal correlations and pathways using in-house bioinformatics pipeline.
Result: Branching evolution was observed as the most common pattern of clonal evolution in 70% MM patients. A few genes were preferentially mutated at diagnosis (e.g., ATR, ALK, BIRC3, TET2) or on progression (e.g., FLT4, JAK2, KMT2A) while others like TP53, NRAS, BRAF were found mutated at both time points. Clonal gains were predominant in NRAS, TP53, BRAF, MAGI3 while clonal losses were common in LAMA1, PTPRF. A drop in %clonality on progression was seen in LOXHD1 while an increase was observed in MAGI3 and TUSC3. Paired clonal mutations such as TP53 + SYNE1, NRAS + MAGI3 and KRAS + TP53 showed significant Pearson correlations. Clonal mutations in FCGBP and FAT3 showed mutual exclusivities. Clonal mutations perturbed KEGG pathways at diagnosis (Lysine degradation, ABC transporters, Adherens junction) while MAPK signaling, apoptosis at progression.
Conclusions: Oncogenic dependencies corresponding to specific mutant gene pairs exist that could impact progression in MM. Since the mutational profiles at diagnosis are different than at progression, a regular monitoring of druggable/actionable gene targets of clinical relevance can aid early and personified treatment of patients.
Acknowledgements: Department of Biotechnology, Govt. of India [BT/PR7136 /MED /30/901/2012; BT/MED/30/SP11006/2015], Department of Science and Technology, Govt. of India [DST/ICPS/CPS-Individual/2018/279(G)] and Multiple Myeloma Research Foundation (MMRF) personalized medicine initiatives (https://research.themmrf.org and www.themmrf.org).

Whole Exome Sequencing Reveals Preferential Loss of Arginine Codons in Multiple Myeloma

Ritu Gupta, Gurvinder Kaur, Lingaraja Jena, Anubha Gupta, Akanksha Farswan, Atul Sharma, Lait Kumar

Introduction: Multiple Myeloma (MM) is genetically a heterogeneous disease with diverse mutations across patients. However, a few patters of gene mutations may aid in risk stratification/prediction of clinical outcomes and merit deep investigations.
Aims & Objectives: The aim of this study was to analyze coding mutations, patterns of amino acid substitutions and their clinical impact in MM.
Materials & Methods: Whole exome sequencing was performed using Nextera Exome library prep kits on malignant plasma cells obtained from 71 MM patients. Mutational variants were called with Illumina Dragen pipeline followed by in-house bioinformatics analyses. Levels of circulating arginine in plasma were estimated with Abcam's L-Arginine assay.
Result: MM patients had somatic nonsynonymous mutations in 106 oncogenes, 148 tumor suppressor genes with a median tumor mutation burden of 1.5. These mutations led to a selective maximal net loss of arginine specific codons. The circulating arginine levels were significantly reduced in plasma of such patients. Kaplan Meier curve analyses showed a significant correlation between no loss of arginine with inferior PFS ($p = 0.012$). Arginine is a key player in cell metabolism and supports growth and survival of malignant cells. If there is no loss of arginine, its continued abundance would tend to favor tumor progression and thus inferior outcomes. The dNS/dS analysis identified a novel potential driver SPANXD (Sperm Protein Associated with the Nucleus on the X chromosome-D). SPANXD is a cancer testis antigen and an attractive target for cancer immunotherapy and warrants further evaluation in MM.
Conclusions: This study has shown a selective net loss of arginine codon usage in MM and its clinical impact on PFS. A targeted deprivation of Arginine in the tumor microenvironment is a potential therapeutic modality under trials in certain cancers and could be explored in MM. Role of SPANXD in MM also needs to be further investigated.

Diffuse Large B-Cell Lymphoma (DLBCL) in an Infant Acquired Through Trans-Placental Spread

Samipa Das, Aaishwarya Dhabe, Karthik Ramakrishnan, Pranay Gurung, Niharendu Ghara, Arpita Bhattacharyya, Reghu K S, Debdeep Dey, Indu Arun, Sushant S. Vinarkar, Asish Rath, Deepak Kumar Mishra, Mayur Parihar

Introduction: Vertical transmission of malignancies from a mother to fetus is a rare phenomenon. The most common cancers that have been reported to be transmitted from mother to fetus are melanoma, hematological malignancies (Leukemia and lymphoma), sarcomas, breast and lung carcinomas. We report an extremely rare presentation of Diffuse Large B Cell Lymphoma in a 7-week old infant, the neoplasm being transmitted from the mother.
Aims & Objectives: To report a case of DLBCL in an infant and demonstrate vertical transmission from the mother using cytogenetic and molecular techniques.
Materials & Methods: The diagnosis of DLBCL was made on lymph node biopsy on morphology using standard immunohistochemistry panel. FISH was performed on formalin fixed paraffin embedded (FFPE) biopsy sample of the infant's tumor using CEN X/Y Dual Color Probe (Zytovision Bremerhaven, Germany). STR loci profiling (D5S818, THO1, vWA and AMEL loci) was performed using DNA extracted from FFPE blocks of both mother and the infant using PowerPlex 16 HS System (Promega Corporation; Cat No: DS2101), followed by capillary electrophoresis and analyzed by Genemapper software.
Result: A 7-week infant delivered at 33 weeks by LSCS presented with axillary and inguinal lymphadenopathy. The mother was diagnosed as rectal DLBCL on peripartum day 3 and died on day 10 after the birth of the infant.
USG guided FNAC of inguinal lymph node suggested a lymphoproliferative disorder and biopsy confirmed the diagnosis of Diffuse large B-cell lymphoma, Non Germinal-Center-B-Cell type (DLBCL, Non-GCB). The mother's tissue blocks were reviewed and showed a similar tumor and IHC profile. A FISH using CEN X/Y probe on the tumor tissue of the infant revealed a XX genotype indicating tumor cells were of female origin and acquired from the mother. The endothelial cells and stromal cells in the biopsy showed a XY genotype. Molecular analysis of short tandem repeats using DNA extracted from tumor tissue blocks of the mother and the infant confirmed the lymphoma to be of maternal origin. The infant died on day 16 of starting therapy.
Conclusions: We report a rare case report of maternal transmission of DLBCL to the infant. Further studies on HLA haplotypes is required to understand why the maternal lymphoma was not rejected by the infant.

High Grade B-Cell Lymphoma (NOS): Two Rare Case Reports

Ankita Pal, Raka Hota

Introduction: High-grade B-cell lymphoma (HGBL) is a newly introduced category in the updated 2016 revision of WHO classification, which primarily replaces "B-cell lymphoma, unclassifiable, with features intermediate between a diffuse large B cell lymphoma (DLBCL) and the Burkitt lymphoma (BL). Currently, HGBL comprises 2 types of lymphomas: HGBL with MYC and BCL2 and/or BCL6 rearrangements and HGBL, NOS. Latter type is a heterogeneous category of clinically aggressive mature B-cell lymphomas that lack MYC plus BCL2 and/or BCL6 rearrangements and do not fall into the category of diffuse large B-cell lymphoma (DLBCL), NOS, or Burkitt lymphoma (BL). These cases are rarely encountered with no clear eitiology and pathogensis. Standard treatment has not been established for HGBL,NOS but the following regimens have been used at the National Comprehensive Cancer Network member institutions like R-CHOP, DA-EPOCH-R & autologous hematopoietic stem cell transplantation.

Aims & Objectives: A rare case report and how to overcome the diagnostic dilemma.

Materials & Methods:

CASE 1

71 year male presented with swelling over right anterior chest wall.Complete blood count revealed pancytopenia with atypical lymphoid cells (?leukemia/lymphoma).FNAC was done outside suggesting malignant lesion ?NHL,?Metastasis. Bone marrow aspiration revealed 40% atypical lymphoid cells which were intermediate in size with high N/C ratio, vesicular chromatin,scant to moderate cytoplasm and very prominent centrally placed nucleoli suggestive of lymphoproliferative disorder. Bone marrow biopsy showed lymphoma infiltration to the marrow.

CASE 2

50 year male presented with generalised lymphadenopathy.Investigations were same as above described.

Result: Immunohistochemistry study revealed strongly and diffusely positive CD20, BCL-2, BCL-6,C-MYC, weakly positive CD10, negative Cyclin D1 & 50–60% Ki 67. Cases were sent for FISH analysis revealing BCL-2, BCL-6, C-MYC rearragement negative. Collaborating all clinical, morphological, immunohistochemical and FISH study, case diagnosed as HGBL,NOS.

Conclusions: HGBL, NOS were highly aggressive tumors associated with short survival rate.High-intensity chemotherapy combined with ASCT may prolong the survival of patients.Further studies should be done to clarify the clinical outcomes, effective treatments, and survival durations of patients with HGBL, NOS.

In the Era of Immunophenotyping and Molecular Diagnostics Does Morphology Play an Essential Role in Differentiating Hairy Cell Leukemia from its Mimics?

Samikshya Thapa, Anupa Khanal, Priyavadhana B, Neha Singh, Arvind Kumar Gupta, Harish Chandra, Uttam Nath

Introduction: Hairy cell leukemia (HCL) accounts for 2% of all lymphoid leukemias. It is characterized by splenomegaly, cytopenias and atypical lymphoid cells with circumferential cytoplasmic projections. While HCL responds well to chemotherapy, the other morphological differential diagnosis such as hairy cell leukemia variant (HCLv) and splenic marginal zone lymphoma (SMZL) do not respond well to HCL therapy, and have a significantly lower survival. Distinguishing HCL from HCLv and SMZL can be challenging as clinical and pathologic findings often overlap.

Aims & Objectives: To analyse the clinical, morphological and immunophenotypic features of HCL, HCLv and SMZL. To evaluate the role of morphological features in differentiating these entities.

Materials & Methods: This was an observational study which included eight cases of HCL and its mimics diagnosed over a 4-year period. The clinical details, and complete blood counts were recorded. The peripheral smear (PS) and bone marrow aspirate (BMA) slides were reviewed for morphological features such as cytoplasmic projections, amount of cytoplasm, nuclear shape and nucleoli. Bone marrow biopsy (BMB) slides were reviewed for cellularity, pattern and percentage of marrow infiltration, and residual hematopoiesis. Flow cytometry was done in 3 cases which confirmed the diagnosis. Immunohistochemistry (IHC) panel of CD20, CD3, CD5, CD10, Cyclin D1, CD11c, CD25, CD103, CD123 and Annexin A 1 was done in other cases.

Result: There were 5 cases of HCL, 2 cases of HCLv and 1 case of SMZL. All cases of HCL and SMZL had pancytopenia while HCLv had normal or increased total counts. Cytoplasmic projections were seen in 60% of HCL, and 100% of HClv. Prominent nucleoli were seen in HCLv (100%) but not in HCL. HCL showed significantly greater marrow infiltration over HCLv. Marrow was hypocellular in 80% of HCL. On immunophenotyping (IPT), HCL expressed CD19, CD11c, CD25, CD103 and CD123 whereas HCLv lacked CD25. Annexin was positive in all cases of HCL while negative in HCLv and SLVL. (Table 1).

Conclusions: Subtle morphological clues on peripheral smear, BMA/BMB play a pivotal role in diagnosing HCL and its mimics. The ability to narrow down the IHC panel based on morphology will be helpful in limited resource settings.

Table 1: Morphological findings in peripheral blood and bone marrow in HCL, HCLv and SMZL.

MORPHOLOGICAL FINDINGS	HAIRY CELL LEUKEMIA (HCL) % (n=5)	HAIRY CELL LEUKEMIA-variant (HCL-v) %(n=2)	SPLENIC MARGINAL ZONE LYMPHOMA (SMZL) % (n=1)
1. CYTOPLASMIC PROJECTONS			
a) Present- Circumferential b) Present- Circumferential and polar	40 (2) 20(1)	 100(2)	 100(1)
b) Absent	40(2)	0	
2. AMOUNT OF CYTOPLASM	Abundant 100(5)	Moderate-abundant 100(2)	Moderate 100(1)
3. NUCLEAR SHAPE			
a) Round	80(5)	100(2)	Predominantly atypical and occasional round
b) Atypical(dumbbell-shaped, irregular grooves, indented)	20(1)	0	
3. NUCLEOLI			
a) Absent	80(4)	0	Conspicuous to inconspicuous
b) Small and inconspicuous	20(1)	occasional	
c) Prominent	**0**	**100(2)**	
4. Percentage of atypical lymphoid cells in a)Peripheral blood b) Bone marrow	 13-87% 13-30%	 46% & 78% 23% & 43%	 22% 15%
BONE MARROW BIOPSY FINDINGS			
1. CELLULARITY			
a) Hypercellular	20(1)	100(1)	0
b) Hypocellular	80(4)		100(1)
2. PATTERN OF INFILTRATION			
a) Sinusoidal	0	100(1)	0
b) Interstitial	40(2)	0	100(1)
c) Diffuse	60(3)	0	0
3. % OF MARROW INFILTRATE			
a) <25	20(1)	0	0
b) 25-50	40(2)	100(1)	100(1)
c) >50	40(2)	0	0
4. RESIDUAL HEMATOPOIESIS			
a) Suppressed	60(3)	0	0
b) Present	40(2)	100(1)	100(1)

Hepatosplenic T-Cell Lymphoma: Immunophenotype, Laboratory Findings and Survival Outcomes

Mohammed Aakif K A, Phaneendra Datari, Divya Meganathan, Gayathri Kuppusamy, Kotteswari Kathirvel, Anu Korula, Vikram Mathews, Arun Kumar Arunachalam

Introduction: Hepatosplenic T-cell Lymphoma (HSTCL) is a rare and aggressive lymphoma, predominantly arises from γδ T cells that is characterized by the proliferation of small to medium-sized T-cells in the sinusoids of the liver and spleen. Diagnosis of HSTCL is challenging owing to its mimicking of infectious diseases with fever, hepatosplenomegaly, increase in liver enzymes and absence of lymphadenopathy.
Aims & Objectives: To evaluate the clinicopathological findings and Immunophenotype results in HSTCL patients.
Materials & Methods: This retrospective data analysis includes 29 patients diagnosed with HSTCL between 2006 and 2021. Clinical and lab findings including immunophenotype were retrieved from the electronic medical records.
Result: Among the 29 patients in the cohort, clinical details were available in 27 patients. The median age at presentation was 33 years with a male to female ratio of 3.7. Fever was the most common presenting complaint followed by abdominal discomfort, fatigue and bleeding. Splenomegaly with or without hepatomegaly was seen in all the patients while palpable lymph nodes were seen in 2 patients, one of whom had co-existing tuberculosis. Median values of Hb, WBC and platelets were 8.9 g/dl (4–12.4), 3500/μl (900–21,750) and 59,000/μl (4000–610,000). Common immunophenotype observed was moderate to bright expression of CD2(83%), CD3(100%), CD7(97%), CD56(65.5%), variable expression of CD8(51%) while CD5(17.2%) and CD57(10%) were predominantly negative. The majority of patients (85.5%) showed TCR- γδ while TCR-αβ was seen in 4/27 patients. Cluster analysis yielded two clusters significantly differing in CD38 positivity. Of the 27 patients, only 12 were treated at our centre of which 10 patients completed 6 cycles of chemotherapy (1 early death and 1 patient yet to complete). Nine out of the evaluated 10 patients failed to achieve a remission after 6 cycles of chemotherapy.
Conclusions: The study highlights the dismal outcomes of HSTCL with existing chemotherapy protocols. The cluster with CD38 positivity stands as a promising subset for targeted therapy with Daratumumab.

Bone Marrow Histomorphological Findings in T-Cell Lymphoma: A Conundrum of 5 Cases

Abhiruchi Sharma, Sarika Singh, Sunita Aggarwal

Introduction: T-cell lymphoma (TCL) is a rare and heterogenous disease entity accounting for 12% of all Non-Hodgkin's lymphomas. Common subtypes include Peripheral T- cell Lymphoma -NOS (PTCL), Angioimmunoblastic T-cell lymphoma (ATCL), Adult T-cell Leukemia/lymphoma (ATLL) & Anaplastic large cell lymphoma (ALCL). PTCL, arising from mature T cells, is a disease of the adults, with M:F ratio of 2:1. Peripheral lymphadenopathy is the initial presentation, due to their aggressive nature and limited treatment options available, these lymphomas generally have a poor outcome. Those presenting with advanced stage show secondary involvement of spleen, bone marrow and liver & a small subset of patients present with paraneoplastic features such as eosinophilia, pruritis and hemophagocytic syndrome. Bone marrow biopsy (BM Bx) acts as pivotal investigation for further workup, staging and at times the only finding in patients with cytopenias showing interstitial, focal or rarely diffuse pattern of tumor cell infiltration. The tumor cells are highly pleomorphic with variation in nuclear shapes and chromatin pattern with non neoplastic reactive infiltrate in background. ATCL shows infiltration pattern of interstitial, paratrabecular or rarely diffuse with small to medium-sized neoplastic lymphocytes having irregular nuclei along with infiltration of lymphocytes, plasma cells, macrophages and immunoblasts in background.
Aims & Objectives: Role of BM Bx findings and immunohistochemistry(IHC) to accurately diagnose and categorize the above discussed pathology.
Materials & Methods: A retrospective study (2018–2022) conducted in the department of Pathology, MAMC. 05 patients were selected from the archives. Peripheral blood films & subsequently bone marrow examination was performed in all cases. BM Bx were further subjected to IHC using CD3, CD4, CD5, CD7, CD8, CD52, CD20, CD30, CD10, CD21, CD15, ALK and EMA. Two cases underwent lymph node biopsy as well.
Result: Out of 05 patients (ranging from 30–70 years) selected for the study, 4/5 had lymphadenopathy & hepatosplenomegaly, 3/5 had cytopenia, 1/5 presented with AIHA and 1/5 showed pruritic scaly lesions all over the body. After thorough microscopic examination of BM Bx and extensive immunohistochemical analysis, 4/5 patients were reported out as PTCL & 1/5 as ATCL. 2/5 patients succumbed to disease during workup owing to their very late presentation. 1 /5 patient. was lost to follow up. 1/5 patient is on chemotherapy and 1/5 patient is free of disease post chemotherapy and is on follow up.
Conclusions: High clinical suspicion combined with early bone marrow examination can help diagnose this condition in time to initiate early and appropriate management and prevent undue mortality.

Waldenstrom Macroglobulinemia with 11q Deletion-A Rarely Diagnosed Entity

Mouli Mishra, Giri R,Sahu N, Mishra P, Senapati U, Bhuyan B

Introduction: Waldenstrom Macroglobulinemia(WM) is a type of lymphoplasmacytic lymphoma (LPL) that is associated with bone marrow involvement and IgM monoclonal gammopathy.WM is an extremely rare neoplasm with an incidence of 3–4 cases per million people per year.7
Aims & Objectives: Here we present a case of Waldenstrom Macroglobulinemia with 11q deletion.
Materials & Methods: A 70-year-old male, presented with weakness and shortness of breath on exertion for 4 months.There was no hepatosplenomegaly. CBC showed anemia(Hemoglobin-5.5 g/dl) and thrombocytopenia(Platelets-1lakh/cumm). ESR-02 mm/1st hour. Serum electrophoresis showed 'M'spike with a level of 4 g/dl. Immunofixation electrophoresis(IFE) identified the 'M' spike as IgM, Kappa. Bone marrow aspiration was hypercellular composed of mostly atypical lymphocytes,few showing plasmacytoid differentiation which constituted 80% & plasma cells accounted for 5% of the bone marrow DC. Trilineage hematopoiesis was decreased. Bone marrow biopsy showed intertrabecular pattern of infiltration by atypical lymphocytes,a small subset of plasmacytoid lymphocytes and few mature plasma cells.On immunohistochemistry, the lymphocytes and plasmacytoid lymphocytes cells showed strong membranous positivity for CD20, focal scattered positivity for CD38, CD138 and negative for CD3, CD5, CD23,CD10. Fluorescence In Situ Hybridisation and the cytogenetic study showed 11q deletion. Polymerase Chain Reaction showed MYD88(L265P) mutation. PET-CT showed low grade metabolism in supradiaphragmatic and infradiaphragmatic adenopathy.
Result: Thus, considering the clinical findings, serum electrophoresis showing 'M'spike, Immunofixation electrophoresis confirming the 'M'spike as IgM kappa, bone marrow aspiration,and biopsy showing lymphoplasmacytoid cells and IHC showing CD20,CD38 & CD138 positive with CD3,CD5,CD10,CD23 negative, the diagnosis of WM with 11q deletion was rendered..Currently the patient has received 6 cycles of chemotherapy and is on complete remission.
Conclusions: This case is presented for its rarity and the panel of laboratory tests which have helped in arriving at the diagnosis.11q deletion in WM is associated with poor prognosis.

T-Lymphoblastic Lymphoma/Leukemia Diagnosed on a Poorly Fixed Lymph Node Biopsy with the Help of Immunohistochemistry: Report of a Challenging Case

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Introduction: Proper tissue fixation is an important prerequisite for accurate histologic diagnosis. Artifacts of improper fixation can lead to diagnostic issues in evaluation of hematolymphoid neoplasms. Necrobiosis is the transition phase between necrotic and viable cells, where antigenicity is preserved in morphologically non-viable cells. Nuclear antigens tend to disappear with cellular degeneration much faster than cytoplasmic antigens.
Aims & Objectives: We worked up a case with a poorly fixed lymph node biopsy which although had poorly preserved morphology, antigens were preserved, and we could arrive at the diagnosis with the help of a panel of immunohistochemical (IHC) stains.
Materials & Methods: A 46-year-old lady presented with bilateral cervical lymphadenopathy, hepatosplenomegaly, and right sided pleural effusion. FNAC was suggestive of high grade Non-Hodgkin lymphoma, peripheral blood smear was normal. Following which excision biopsy was done, but unfortunately the specimen was transported to the laboratory without formalin. Owing to this, the H&E section had poorly preserved morphology. To resolve the diagnostic and therapeutic issues, we proceeded with IHC, which was conclusive.
Result: H&E section showed few preserved secondary follicles in the periphery. Morphology of the cells in other areas couldn't be made out. This was communicated to the clinician, including the need for repeat biopsy. The patient was very ill and empirically started on CHOP and the nodes had decreased in size. To ascertain the lineage of the cells, we decided to proceed with IHC in the biopsy. First panel with CD20 and CD3 stained only the reactive B and T cells respectively. Further panel of IHC revealed a neoplastic proliferation which were positive for TdT, CD34, CD7, with high Ki67 and negative for other B, T and myeloid makers. Thus, a diagnosis of T- lymphoblastic lymphoma/leukemia was made.
Conclusions: Even though poor fixation may lead to poorly preserved morphology, and loss of antigens, it is always worth doing a panel of IHC as antigenicity may be preserved in spite of morphological degeneration.

Circulating Normal and Tumor Plasma Cells in Multiple Myeloma: Insights Into Expression Profiles of Surface Markers At Baseline and During Course of Therapy

Aishwarya Dash, Pratibha Suku, Parveen Bose, Nabhajit Mallik, Sreejesh Sreedharanunni, Man Updesh Singh Sachdeva, Aditya Jandial, Pankaj Malhotra

Introduction: Circulating plasma cells are a distinct sub-population of plasma cell. Differentiating circulating normal and tumor plasma cells (CNPC & CTPC) in patients with multiple myeloma (MM) require panel of surface markers. Scant literature is available on expression profiles of markers on CNPC and CTPC, and on their stability in due course of therapy.
Aims & Objectives: To analyze and compare the immunophenotypic profile of circulating normal and tumor plasma cells and evaluate the stability of markers over the duration of therapy.
Materials & Methods: Treatment naïve patients of MM (> 18 years) were enrolled. Six millilitres of anticoagulated peripheral blood was collected before & after 60 days of the treatment. Multicolor flow cytometry was carried out using panel of antibodies i.e. CD45, CD38, CD138, CD19, CD81, CD27, CD56, CD200 & CD28, cytoplasmic-kappa & -lambda. Median fluorescence intensities (MFI) of surface-markers at 2 time points were noted and statistically compared.
Result: Twenty-one patients of MM were enrolled with mean age of 58 (range 34–82) years. All patients received bortezomib-based therapy.
CNPCs were present in all patients, both at baseline ($0.170 \pm 0.264\%$) and 60 days ($0.074 \pm 0.107\%$). CTPCs were found in 16 patients at baseline ($0.764 \pm 2.14\%$) and 3 patients at day-60 ($0.044 \pm 0.057\%$).

Notably, in contrast to PCs in bone marrow, CD138 expression on CNPCs was very low. Interestingly, CD138 expression was bright on CTPCs.

CD19, CD45. CD27 & CD81 showed significantly low expression in CTPCs compared to CNPCs ($p < 0 >$

After 60 days of therapy, CD19, CD27, CD81, CD56 and CD138 did not show significant alteration in expression on CNPCs, whereas the expression of CD45 was significantly higher ($p = 0.014$).
Conclusions: CD138 had low expression on CNPCs, and hence cannot be used alone for their identification.
CD19, CD45, CD138, CD27, CD81 & CD56 are good for differentiating CNPCs from CTPCs.

All the markers, except CD45, remained stable in their expression on CNPCs after 60 days of therapy and can be used during the course of therapy to evaluate treatment response.

The Load of Circulating Tumor Plasma Cells in Patients Of Multiple Myeloma is Associated with Gain of Chromosome 1q21

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Introduction: The role of circulating tumor plasma cells (CTPC) in prognostication and monitoring of plasma cell neoplasms has recently been highlighted in some studies. The load of CTPCs in patients of multiple myeloma (MM) correlate with prognosis. Sparse data exists on association of CTPCs with cytogenetic abnormalities and other prognostic factors in MM.
Aims & Objectives: To evaluate the association of load of CTPCs with cytogenetic abnormalities and other prognostic factors in patients of MM.
Materials & Methods: Twenty-one treatment naïve patients of MM (> 18 years) were enrolled. CTPCs were evaluated using flow cytometric immunophenotyping. Six ml EDTA-anticoagulated peripheral blood, at diagnosis & after 60 days of the treatment, was processed and stained with panel of antibodies i.e., CD45, CD38, CD138, CD19, CD81, CD27, CD56, CD200 & CD28, cytoplasmic-kappa & -lambda. Number of CTPCs were calculated and compared with cytogenetic abnormalities, and other parameters like, lytic lesions, and levels of serum albumin, β2-microglobulin, M-band, and RISS risk-category.
Result: The mean age of patients was 58 (range 34–82) years, with M:F ratio of 3.2:1. CTPCs found in 16 patients at baseline, with mean of 0.764% (range, 0.0013 to 8.6%). After 60 days of therapy, 3 patients had CTPCs with mean of 0.044% (range, 0.003 to 0.11%). There was a significant association of higher CTPCs with presence of 1q21 gain. No association was noted with presence of Tp53 del, Tp53 mutation, Chr1p32 del, IgH::FGRF3, IgH::MAF, lytic lesions, and

levels of serum albumin, β2-microglobulin, M-band, and RISS risk-category.'

Conclusions: CTPCs were noted at baseline in majority of patients of MM (76%). The load of CTPCs was associated with presence of 1q21 gain, but not with other known prognostic factors. The pathobiology of this association needs to be studied for better understanding of disease progression.

Mott Cell: Predominant Myeloma: A Visual Treat on Morphology

Lalita Jyotsna Prakhya, Sunita Sharma

Introduction: Myeloma cells show a varied morphological spectrum, ranging from normal-appearing forms to atypical morphology. The immunoglobulin inclusions in cytoplasm also impart vivid appearances to the malignant plasma cells.

Aims & Objectives: Case report: A 72-year old male presented with chronic fatiguability and on routine tests, Complete blood counts revealed pancytopenia, for which further evaluation was carried out.

Materials & Methods: Bone marrow aspiration and biopsy was done and based on the findings, Serum Protein Electrophoresis was advised.

Result: The hemogram revealed a hemoglobin of 5.3 g/dl, WBC - 3.05X103/μL and platelet count- 53 X 103/μL. Bone marrow aspiration smears showed hypercellular particles with plasmacytosis. Plasma cells comprised 40% of the marrow cells, with predominantly mature-appearing Mott cell morphology, packed with small spherical inclusions, and only occasional immature forms. No plasmablastic forms were seen on the aspirate smears. Erythroid series showed normoblastic reaction, myeloid series showed normal maturation. Occasional megakaryocytes were seen. A serum protein electrophoresis revealed M-spike of 8.3 g/dl. Bone marrow biopsy showed bony trabeculae enclosing hypercellular marrow spaces which showed a diffuse infiltration by Mott cells and occasional plasmablasts. Erythroid, myeloid and megakaryocytic series were suppressed. Immunohistochemistry- Positive for CD138, CD 56 and showed kappa restriction. A diagnosis of plasma cell neoplasm (multiple myeloma) was given.

Conclusions: This case is being presented for the peculiar finding of diffuse Mott cell morphology in a case of myeloma.

Diagnostic Relevance of New Markers in Evaluation of T NHL

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Introduction: T-cell non-Hodgkin's lymphoma (T-NHL) is a heterogeneous group of aggressive NHL arising from T-cell and NK-cell subsets accounting for approximately 10%–15% of all NHLs. The diagnosis and subtyping of T-cell/NK-cell NHL (T/NK-NHL) heavily rely on a multifactorial approach that includes clinical presentation, morphology, immunophenotype, and chromosomal abnormalities.

Aims & Objectives: To compare the difference in expression newer markers in different subtypes of T NHL.

Materials & Methods: The study includes 26 samples of T NHL diagnosed by FCM between April 2021- September 2022 and 6 control samples. Lymphoma screening tube followed by two additional tubes (T-NHL and T/NK CLPD) were processed for all the samples with the following markers: CD2,CD3,CD4,CD5,CD7,CD8,CD10,CD16,CD25,CD26,CD34, CD38,CD45,CD52,CD56,CD57,CD94,TRBC1,PD1,CXCR5,TCRαϖβ,TCRγδ,HLADR, CD19,Kappa and Lambda. Samples were acquired in BD FACSLyricTM flow cytometer and analysed by BD FACSSuiteTM software.

Result: Based on the immunophenotype, samples were subtyped as follows: HSTCL (n = 10 including 2 patients serially evaluated at 4 timepoints), T-LGL (n = 4), T-PLL (n = 4), Sezary syndrome (n = 2), PTCL-NOS (n = 3), AITL (n = 1), ALCL (n = 1) and NK/T lymphoma (n = 1). Marker expression was evaluated in terms of intensity (bright/moderate/dim/heterogenous/negative) and the median fluorescence intensity (MFI). Significant difference in MFI were observed in specific T NHL subtypes compared to other cases and controls. Presence or absence of TRBC1 served as a reliable marker of clonality. Serial evaluation of the markers showed that the expression remained consistent at all the time points in both the samples.

Conclusions: The study highlights the clinical utility of the evaluated markers in diagnosis of T NHL.

Multiple Myeloma & Cryoglobulinemia: Case Report

Mariya, Sruthy V, Bobby G, Roshna P, Reshma, Jesina, Bonnie A G, Priya Prasad, Chepsy C Philip, Elsa John

Introduction: Cryoglobulinemia is a rare disorder characterized by the presence of abnormal immunoglobulins in the blood that precipitate in the tissues causing inflammation and tissue damage. It often occurs in association with diseases such as autoimmune or infectious diseases. Only few cases of cryoglobulinemia associated with multiple myeloma has been described.

Aims & Objectives: Only few cases of cryoglobulinemia associated with multiple myeloma has been described. We report the case report of a 62 year old male patient diagnosed with a relapsed Multiple myeloma, who rpesented with pancytopenia and refractory bleeding from the gums.

Materials & Methods: We report the case report of a 62 year old male patient diagnosed with a relapsed Multiple myeloma, who presented with pancytopenia and refractory bleeding from the gums. There were no other evidence of bleeding. He was diagnosed with Multiple myeloma since 2018 elsewhere and he had recieved CY-BOR-D regienmen and he was on Lenalidomide maintenance. He showed some generalized tiredness and fatigue and hence changed to Pomalidomide maintenance. He presented elsewhere with bleeding gums which was not resolved despite empiric transfusion support and attmempts to achieve local hemostasis. His blood reports showed Pancytopenia.

He underwent a detailed examination and investigations.His reports were suggesttive of a progressive disease with SPEP report showing a thick M band with a conc. of 1.07 g/dl, Beta 2 Microglobulin-16639 ng/mL, Serum free light chain showed an increased Lambda level (2480) and a ratio of 0.002, Immunophenotyping identified the M spike as IgG, Lambda. He received 3 pint PRBC and 4pint RDP and he was started with KPD Regimen for this primary disease.

In view of atypical bleeding possibility of hyperviscocity and spurious cyteopnia secondary to a potential cryoglobulinemia was considered.

Result: The blood sample was centrifuged at 1500 g for 15 min and plasma was separated. The plasma was stored at 4–8 °C. A control sample was also kept alongside. The patient's plasma at 24 h showed white gel like precipitate, thus confirming the presence of cryoglobulins.

Conclusions: In conclusion, cryoglobulimeia in multiple myeloma is only rarely reported. Treatment recommendations include plasmapheresis and treatment of the underlying myeloma.Early idenitification with appropriate treatment can mitigate fatal complications.

Unusual Presentation of Waldenstrom Macroglobulinemia

Saswati Das,Tapaprakash Behera, Arpita Pandia, Pallavi Bhuyan, Kalpalata Tripathy, Lity Mohanty

Introduction: Waldenstrom macroglobulinemia (WM) is a rare and slowly progressive disorder, a variant of lymphoplasmacytic lymphoma. WM presents usually with constitutional symptoms, organomegaly, cytopenia and hyperviscosity syndrome. This neoplasm is composed of small lymphocytes, plasmacytoid lymphocytes and plasma cells that typically involves the bone marrow with IgM paraprotein in the serum.
Case report: We report a case of 67 years male presented with anemia and fatigue, no organomegaly or lymphadenopathy. CBC, Bone marrow aspiration, Bone marrow biopsy, IHC, along with serum protein electrophoresis was done. CBC of the patient revealed anemia. A diagnosis of WM was given after evaluating the bone marrow morphology, IHC and presence of monoclonal IgM in the serum.
Conclusion: This case is unusual because patient lacked the common clinical features. A thorough clinical and hematological workup including S.proteinelectrophoresis, BM study and IHC helps in distinguishing WM from other lymphoma and plasma cell dyscrasias.

Haemophagocytic Lymphohistocytosis Complicating Systemic Sarcoidosis

Suprasidha Mohanty, Tapaprakash Behera, Pallavi Bhuyan, Pranati Mohanty, Lity Mohanty

Introduction: Hemophagocytic lymphohistocytosis(HLH) is a reactive condition having familial form (presents early in life) and sporadic forms(may appear at any age). It is characterized by cytopenia and sign and symptoms of systemic inflammation, which occurs due to macrophage activation hence refered to as macrophage activation syndrome(MAS).
Case report: A 24 year old male presented with right orbital and left parotid swelling with multiple skin nodules over the abdomen associated with continuous high grade fever for a month. He developed progressive cytopenia along with increase in CRP and liver enzymes. He was treated with steroids and antibiotics as required. Skin nodules and liver biopsy microscopically showed granulomas. Bone marrow aspiration showed hemophagocytosis, thereby helping in diagnosis of a rare case of HLH complicating systemic sarcoidosis.
Conclusion: 1. Hemophagocytotic lymphohistocytosis has rarely been reported as a complication of systemic sarcoidosis which if suspected early can improve management and prognosis.
2. Sarcoidosis can occur in absence of pulmonary involvement.

Lymphoma and Myeloma: Laboratory

Evaluation of Expression of KI-67, P53 By Immunohistochemistry and Their Correlation with Microvessel Density in Cases of Multiple Myeloma

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Introduction: Multiple myeloma (MM) is the second-most common haematological malignancy. It accounts for 1% of all cancers and approximately 10% of all haematological malignancies. Diagnostic laboratory workup is done keeping in view international myeloma working group diagnostic criteria of MM. Durie-salmon staging, international staging system (ISS) and revised—ISS are used for staging and prognostication of MM. Recently, three markers which can be assessed on paraffin blocks, viz, microvessel density (MVD), Ki-67 and p53 have attracted focus to be used as prognostic markers.
Aims & Objectives: The present study aimed to evaluate expression of Ki-67, p53 in bone marrow biopsies and it's correlation with MVD in patients with MM.
Materials & Methods: 74 patients were enrolled in this study between 2017–2022, 59 with multiple myeloma and 15 with non-malignant disease as controls. Proliferative activity was analyzed by Ki67, TP53 deletion was analyzed by p53 and MVD was assessed by CD34 and compare between the two groups. In myeloma patients correlation between Ki67, p53, MVD and other prognostic factors was assessed by Pearson correlation coefficient.
Result: According to ISS, 31 patients were of stage I,11 of stage II and 17 of stage III. Ki67, MVD and p53 did not show any correlation with each other. MVD/10hpf was significantly higher in myeloma patients (mean-5.0%) than controls (mean-0.107%). Ki67 was also significantly higher in myeloma patients (mean-8.1%) than controls (mean-1.80%). p53 staining was not seen in controls and in myeloma patients (mean-13.03%). MVD showed significant correlation with ISS (p-value < 0.0001) and it increases as stage increases. MVD showed statistically significant positive correlation with β2 microglobulin. Ki67 showed statistically significant positive correlation with serum creatinine (p- value 0.033). p53 did not show any correlation with biochemical and haematological parameters. (Image is Attached in below section Please consider it as Result image and title for images is) CD34 immunostaining in cases revealing increased MVD (X400).
Conclusions: MVD is an indicator of aggressiveness in MM and it is significantly correlated with ISS stage and MVD increases as stage increases. Therefore, it can be used as a prognostic marker and for risk stratifications of patients. The routine determination of this parameter helps to identify patients with aggressive disease who may benefit from intense therapy.

Role Of LAIR1 (CD305) in Flow Cytometric Detection of Occult Bone Marrow Involvement in Non-CLL B-Cell Non-Hodgkin Lymphoma

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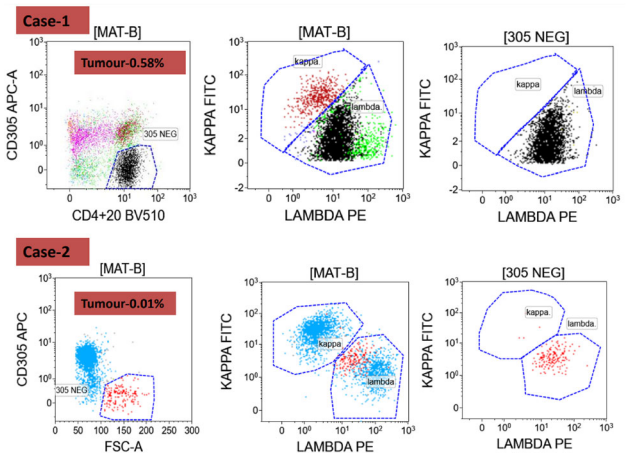
Introduction: Staging of B NHLs is crucial as it provides definitive evidence of stage of disease and is important for making a decision regarding treatment strategy. Bone marrow involvement is a vital part of staging B-NHL. We hypothesized that CD305, also known as LAIR1 (leukocyte immunoglobulin-like receptor-1), is a robust marker for detecting occult bone marrow involvement. LAIR expression varies during various stages of B cell differentiation.
Aims & Objectives: To decipher the role of CD305 expression in B-NHL tumor cells and normal mature B cells for detecting occult bone marrow involvement.
Materials & Methods: CD305(BV78, clone DX26) was incorporated in the lymphoma screening tube used for assessment of bone marrow(BM) involvement as a part of B-NHL staging between January 2020 and December 2021. Occult bone marrow involvement was defined as B-NHL involvement with $< 10\%$ lymphoma cells. Multicolor Flow cytometric immunophenotyping(MFC) was performed using a 13-color panel on DxFlex flow-cytometer. Data was analyzed using Kaluza-V2.1-software.
Result: We studied BM samples from 1084 patients. Of them, 148 cases were clearly involved by morphological/immunophenotypic evaluation. Of the remaining, 172 cases did not reveal morphological involvement and were detected on MFC independently with a median tumor burden of 1.05(0.005–89). Median age was 57 years(range 7–80). These included CD5 positive lymphomas 35/172(20.3%), CD10 positive lymphomas 77/172(44.7%) and CD5, CD10 double-

negative lymphomas 60/172, (34.8%). 149/172 (86.6%) cases revealed occult BM involvement. In 93/149 (62.4%) cases, B-NHL cells were identified using the expression-pattern of CD5, CD10, CD11c, CD20, CD38, CD45, and Forward scatter in the background of normal B-cells. In the remaining 48/149(32.2%) cases, the tumor population was identified using only CD305 negative expression. Median(range) of tumor burden was 0.59% (0.016–9.7%). Overall, CD305 was downregulated in 155/172(90.1%) cases. CD305 was indispensable in the detection of occult BM involvement in 56(37.6%) cases which, lacked other immunophenotypic abnormalities and was helpful in 99/172(57.6%) samples.

Conclusions: CD305 is a crucial marker in detecting bone marrow involvement in cases of B-cell NHL by MFC, particularly CD5 and CD10 negative lymphomas where detection of minimal BM involvement would be challenging without the incorporation of CD305 in the lymphoma screening panel. It also provides an aberrant expression as an additional abnormality in a significant proportion of samples and provides supporting evidence in the detection of minute tumor populations.



Flow Cytometry Based Study Of T-Cell Chronic Lymphoproliferative Disorders: An Experience From A Tertiary Care Center

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Introduction: Unlike the more common B-CLPDs, there is no specific immunophenotype (FCM) signature that is diagnostic of a clonal T-CLPDs. Immunophenotypic criteria that are helpful in the diagnosis of T-CLPDs include T-cell subset antigen restriction, anomalous T-cell subset antigen expression, loss/under expression of one of the pan T-cell antigens, a precursor T-cell phenotype, and expression of aberrant markers. FCM has an added advantage of ability to help in early diagnoses/detection of T-CLPDs.

Aims & Objectives: To analyse the immunophenotypic patterns of T-CLPDs by Flowcytometry.

Materials & Methods: We retrospectively analyzed FCM data of bone marrow, peripheral blood and body fluid samples for 17 cases of T-CLPDs from January 2018 to August 2022. The T-CLPD panel included antibodies against CD45, CD3, CD2, CD5, CD7, CD4, CD8, CD16, CD56, CD57, CD1a, TCRαβ and TCRγδ. A cyto-morphological assessment was done in parallel in all the cases.

Result: The median age was 46.5 years with a male predominance (M/F, 13/4). Four patients (23%) had adenopathy and three (18%) had mild-to-moderate splenomegaly at presentation; ten (59%) had erythematous skin lesions. FCM showed mature T-lymphocytes with a predominant CD4 + immunophenotype(n = 7). 5 cases displayed a CD8 + immunophenotype. CD4 + /CD8 + and CD4-/CD8- cases constituted 1 and 4 cases respectively. Complete loss of CD7 expression found in six cases and partial loss noted in two cases. Negative/partial loss of CD5 expression found in five cases. CD7 and CD5 bright expression compared to normal T-cells was seen in only one case. Only a single case showed partial loss of CD2. CD57 was expressed in T-LGL cases(n = 3). CD16 was positive in two of the T-LGL cases. CD56 was positive in one case each of T-LGL, CLPD-NK and HSTCL. Majority of the cases were TCR-alpha/beta(n = 14). TCRG data was available in 7 cases and all of them showed clonal peaks.

Conclusions: FCM features that are most suspicious for a T-CLPD include complete loss of one or more pan-T antigens; altered expression pattern of more than two pan-T antigens in conjunction CD4/CD8 dual-positivity or dual-negative expression. It may also be difficult to distinguish reactive T-cell proliferations from T-CLPDs, where a TCRG rearrangement study may help in reaching a diagnosis.

Renumeration Of T Regulatory Cells In Precursor And Mature B Lymphoid Neoplasms In Adults And Its Correlation With Clinical Stage Of Disease

Bulusu Divya, Rajesh Kumar Bhola, Debahuti Mohapatra, Ripunjay Mohanty, Priyanka Samal, Soumya Surat Panda

Introduction: T regulatory cells (Tregs) play an important role in immune homeostasis by inhibiting the immune response to self or non-self antigens. Tregs counteract tumour immunity and may be responsible for disease progression & poor outcome. An emerging body of evidence points out that Tregs not only inhibit tumour-specific T cells but may also have a role in suppressing the progression of the B-cell tumour.

Aims & Objectives: A prospective observational study has been carried out to enumerate circulating T regulatory cells by Flow cytometry in precursor and mature B lymphoid neoplasms of adults and to correlate with clinical stage of B lymphoid neoplasms.

Materials & Methods: The peripheral blood samples has been collected from 20 controls & 56 cases who have been diagnosed as precursor B acute lymphoblastic leukemia or mature B cell neoplasm including plasma cell neoplasm. A flow cytometry assay was performed using a CD45, CD3, CD4, CD8, CD25, CD127, FoxP3, CD45RA & CD45RO antibodies to elicit different T cell subsets including T regulatory cells with its naive, memory & activated subsets.

Result: A total of 56 cases were studied amongst which 23 cases were plasma cell neoplasms, 15 cases were B-ALL, 6 cases were CLL, 7 cases were diffuse large B cell lymphoma, 2 cases of follicular lymphoma, 2 cases of mantle cell lymphoma and 1 case of marginal zone lymphoma. Heterogeneity was observed among different B cell neoplasm with higher numbers of circulating Tregs are observed in more aggressive neoplasm.

Conclusions: Tregs may play a role in modifying immune responses in patients with lymphomas and may be useful in immunotherapy and new anti-lymphoma strategies involving depletion of Tregs.

Comparison Of Lymph Node Sampling Through Complete Excision Versus Core Needle Biopsy: Focussing On Diagnostic Accuracy And Feasibility For Interpretation

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Introduction: Lymph nodes are one of the most commonly biopsied tissue for diagnosis of various disorders. In recent times, core biopsies

are increasingly being preferred over whole node excision by clinicians. Proper interpretation of lymph nodes requires assessment of architectural patterns in addition to cell morphology, a feature available only in excision biopsy. A core biopsy poses inherent challenges since architectural pattern cannot be assessed, and focal pathologies may completely be missed. Review of literature documents limited studies addressing the issue of accuracy of reports with core versus excisional lymph node biopsy.

Aims & Objectives: To assess the diagnostic efficacy of core biopsy and complete excision biopsy in diagnosing lymphnode pathologies.

Materials & Methods: This was a retrospective study conducted on lymph node biopsies, received over four-year period. Lymph node biopsies were categorized into two groups, core biopsies and excision biopsies groups and were analyzed based on routine Hematoxylin and eosin stain and whenever applicable with immunohistochemistry(IHC). Each group was evaluated to obtain percentage of cases diagnosed on morphology alone/morphology with IHC. Analysis of cases in each groups were done to determine the percentage of cases lacking a definitive diagnosis even after performing ancillary tests. The various challenges faced while interpreting core biopsies were noted.

Result: A total of 554 lymph node biopsies were received which included 182 (33%) core biopsies and 372 (67%) excisional biopsies. Histopathological examination revealed 219, 274 and 61 cases to be benign, malignant and non-diagnostic respectively. Diagnosis could be made on 125/182 core biopsies and 368/372 excision biopsies with a diagnostic sensitivity of 68% and 99% respectively. Definitive diagnosis could not be made on 31.3% of core biopsies, and 1.07% of excision biopsies, even after performing ancillary tests. Lymph node core biopsies were more prone to fragmentation, crushing artefact, cellular distortion, and shrinkage, thereby challenging the interpretation.

Conclusions: Complete lymph node excision reflected better efficacy and was found to be a superior sampling technique as compared to core biopsies in rendering an accurate final diagnosis. The former technique has a better feasibility and poses minimal complexity in interpretation as compared to latter. Procedural and tissue artefacts limits interpretation in core biopsies.

Key words: core biopsy, efficacy, excision biopsy, lymphnode, sampling technique,

Bleeding Diathesis Due To Multiple Acquired Factor Deficiencies In Plasma Cell Dyscrasias: A Rare Presentation

Immanuel Ratan Stephen, Abhijit Lakshmanan, Febe R Suman, Krishnarathnam

Introduction: Multiple myeloma is a clonal plasma cell proliferative neoplasm with excessive monoclonal paraprotein production leading to end organ damage. Bleeding diathesis not responding to conventional therapy with multiple acquired coagulation factor abnormalities with underlying plasma cell dyscrasia on initial presentation is a rare phenomenon.

Aims & Objectives: To present a rare case report of bleeding diathesis with multiple acquired coagulation factor deficiencies in multiple myeloma.

To emphasize the importance of Thrombin time, fibrinogen, factor V and factor X assay in a patient with multiple myeloma.

Materials & Methods: Demographic and clinical data were retrieved from hospital laboratory information system while outcome data was provided by consultant hematologist.

Coagulation assay was performed in Sysmex CS-2400 coagulation analyzer.

Result: We present a case of a 60 year old male with hematuria, epistaxis and per rectal bleeding with noother significant complaints. Patient was on regular medication for systemic hypertension. Initialhematology and biochemical workup showed anemia, thrombocytopenia,prolonged PT,aPTT and TT.

F with elevated serum creatinine and reversal of albumin-globulin ratio. Further workup showeddecreased fibrinogen, factor V and factor X while increased von-Willebrand factor activity was noted.-Bone marrow aspirate and biopsy showed increased plasma cells with immunohistochemistryconfirming lambda light chain restriction. Serum immunoelectrophoresis showed the presence of M-band of 1.6 g and serum immunofixation showed IgG lambda monoclonal gammopathy. Abdominal fat pad biopsy was negative for amyloid deposition. Normal liver function tests ruled out dysfibrinogenemia secondary to hepatic dysfunction. Tests for autoimmune antibodies and lupus anticoagulant were also negative. Patient was initially transfused 2 units of packed RBC and 4 units of fresh frozen plasma but showed minimal response. Upon diagnosis of multiple myeloma started on CyBor-D chemotherapy to which the patient responded well.

Conclusions: Paraproteins released by myeloma cells can induce acquired factor deficiency.

Coagulation factor assays are useful in the diagnosis of multiple myeloma induced acquired factor deficiency.

CD5 Positive Hairy Cell Leukemia: A Rare Entity That Highlights The Need For A Comprehensive Phenotyping Panel Upfront

Trupti Shetty, Heena Satam, Amar Dasgupta, Huma Iqbal, Mehjabeen Sheikh

Introduction: Hairy cell leukemia (HCL) is a smoldering B cell neoplasm characterized by medium sized lymphoid cells with 'hairy' cytoplasmic projections infiltrating the spleen, the lymph nodes and the bone marrow. HCL is a CD5-negative, CD10-negative B-cell neoplasm. However, an occasional case of CD5 + HCL with a documented BRAF V600E mutation has been reported.

Aims & Objectives: We report a case of CD5 positive HCL with BRAF V600E mutation. To our knowledge, this is the only case report of a documented BRAF V600E mutated CD5 + HCL in India.

Materials & Methods: A 46-year-old male who complained of mild abdominal pain was found to have moderate splenomegaly. Complete blood count (CBC) was performed on an automated Hematology analyser (Horiba, Japan). Multicolour flow cytometric immunophenotyping performed on DxFlex Flow Cytometer (Beckman Counter, USA) using a large panel of antibodies directed against B- and T-lymphoid markers including CD11c, CD25, CD103 and CD200. BRAF V600E mutation was looked for by molecular analysis.

Result: CBC showed the hemoglobin of 17.3 g/dL, a white blood cell count of 8.8 × 10^3/cumm, with spurious monocytosis (46%) reported by the hematology analyser, and thrombocytopenia (platelets 56 × 10^3/cumm).

The peripheral smear showed atypical lymphoid cells with low N:C ratio, folded nuclei with open chromatin and "hairy" cytoplasmic projection associated with monocytopenia. Bone marrow examination was not performed.

Multicolour flow cytometric immunophenotyping revealed that the neoplastic lymphoid cells were CD5 + , CD11c + , CD19 + , CD20 + , CD23 + , CD25 + , CD103, CD200, sIgM + , sIgD + and showed lambda light chain restriction. Molecular analysis showed BRAF V600E mutation.

Conclusions: Conventionally, HCL is classified as a CD5 negative B chronic lymphoproliferative disorder (CLPD) to distinguish it from B-chronic lymphocytic leukemia (CLL) and mantle cell lymphoma. The rare cases of CD5 positive HCL highlight the heterogeneity among HCL cases. Identification of 'hairy' cells in HCL is often

missed and/or misdiagnosed as monocytes at the level of CBC leading to a delay in diagnosis. Hence, there is a need to include HCL markers (e.g. CD11c, CD103, CD123 and CD25) in the primary reagent panels used for the diagnosis of CLPD for an early diagnosis of HCL. Cases like the one described here have an immunophenotype that overlaps that of B-CLL (CD5 + , CD19 + , CD20 + , CD23 + , CD200 +) and needs to be distinguished from the latter entity with the help of molecular studies.

CD49d Positivity In B-Chronic Lymphocytic Leukemia Is Low In Indian Patients: Experience At A Reference Laboratory

Amar Dasgupta, Trupti Shetty, Huma Iqbal, Mehjabeen Sheikh

Introduction: Expression of CD49d by the leukemic cells in B-Chronic Lymphocytic Leukemia (CLL) has been linked to poor prognosis. CD38 and Zap70, the other independent prognostic markers in B-CLL are inferior to CD49d in this respect. Interestingly, anti-CD49d antibody is not included in most immunophenotyping panels used in India.
Aims & Objectives: To assess (i) the incidence and level of positivity of CD49d in B-CLL cases in order to determine its usefulness in chronic lymphoproliferative disorders (CLPD) immunophenotyping panel, and (II) to find out correlation between CD49d and CD38 expression, if any.
Materials & Methods: Flow cytometric immunophenotyping of 53 cases of chronic lymphoproliferative disorder (CLPD) was performed using an eight-colour antibody panel directed against CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD19, CB20, CD23, CD38 (clone LS198.4.3), CD45, CD49d (clone HP2/1), CD103, CD200, FMC7, surface(s) IgD, sIgM, kappa and lambda light chains. Cases with = / > 20.
Result: There were 25 B-CLL cases (CD5 positive, CD19 positive, CD20 dim, CD23 positive, CD200 positive, kappa or lambda light chain restricted). The incidence of CD49d positivity was low (12%; 3/25). Percent positive cells ranged from 20 to 60. CD38 positivity was also low (16%; 4/25). One of the 3 cases was positive for both CD49d and CD38. No correlation was found between expression of CD49d and other markers included in the reagent panel.
Conclusions: The low incidence of CD49d and CD38 positivity observed by us among the B-CLL cases is at variance with the incidence of CD49d positivity (57%) and CD38 positivity (45%) reported in a recent study. This difference could reflect either a biological difference among B-CLL cases in different geographies and/or be due to a difference in the antigenic determinant identified by the different clones of monoclonal antibodies used in different studies, e.g. CD49d—clone 9F10; CD38—clone HB7 were used in the study quoted above. These possibilities will be verified through an extended, larger study.

Myeloproliferative Neoplasm (Total 28 Abstracts)

A Rare Phenomenon Of Lineage Switch From Myeloid Blast Crisis Of Chronic Myeloid Leukemia To B Lymphoblastic Leukemia

Renuka Verma, Monika Gupta, Sunita Singh

Introduction: Blast crisis of chronic myeloid leukemia (CML-BC) may involve any hematopoietic lineage.
Aims & Objectives: In a minority of cases, blast lineage may switch from myeloid to B- lymphoid, especially in cases of imatinib-resistant CML-BC.
Materials & Methods: A 39 years old female presented with generalized weakness and fever. On examination, patient was anaemic with hepatosplenomegaly. On peripheral blood film, TLC was 23,000/cmm, with myeloid bulge, 26% blasts, basophilia and 3.5 lakhs/cmm platelet count, a diagnosis of CML-BC was made. On flow cytometry, CD 34, CD 4, CD 7, CD117, CD 13, CD 33, MPO, and HLA-DR were positive suggestive of CML with myeloid blast crisis. On cytogenetics, BCR-ABL1 kinase mutation was negative.
Treatment with Imatinib was started and patient was followed up regularly. Six months later, patient was found to be resistant to imatinib. On PBF, total leucocyte count was 45,000/cmm, with 76% blasts and 3.8 lakhs/cmm platelet count. On HRCT chest, there was leukemic infiltration of bilateral lung parenchyma. On flow cytometry, CD 34, CD 19, CD20, CD 4, CD 7, CD117, CD 13, HLA-DR, CD 79a and tdT were positive suggestive of CML with B-lymphoid blast crisis. DCVP Chemotherapy with Dasatinib was started which diminished the number of blasts.
Result: Blast lineage switch in CML-BC has been shown to be related to clonal selection, interaction of oncogenes, anti-oncogenes, other abnormal genes and transformation of multipotent progenitor cells during conventional chemotherapy.
Conclusions: Combined efforts of morphology, cytochemistry, flow cytometry and genetic analysis are required for definite diagnosis of patient and further research is needed to assess the frequency, treatment, and prognosis of CML-BC patients with lineage switch.

Chronic Myelomonocytic Leukemia: A Case Report

Khevna Kansara, Zalak Parmar, Kailash Inaniya, Faruq Mulla, Sanjay Chaudhari

Introduction: Chronic myelomonocytic leukemia (CMML) is a clonal stem cell disordercharacterized by persistent monocytosis along with myelodysplasia and/or myeloproliferation. Diagnostic criteria include persistent monocytosis ($\geq 1 \times 10^9$/L), monocyte count of $\geq$ 10% of white blood cells count in peripheral blood, absence of another myeloproliferative syndrome and acute leukemia. Also, < 20% blasts (including myeloblasts, monoblasts and promonocytes) in peripheral blood and bone marrow with morphologic dysplasia involving at least one myeloid lineage.
Aim & objectives: To study a case of CMML with heterogenous presentation.
Methods: 70-year-old male patient came to skin OPD with complaints of multiple hyperpigmented papules and nodules,few with crusting presents over chest, abdomen and back. He also had a swelling and redness in right leg for 10–14 days. Incidental finding of leucocytosis was observed with total count of 111.4×10^3/µl. The patient was admitted for further evaluation.
Results: Peripheral blood findings were- Total count: 165×10^3/µl, Hb:7.6 g/dl, Platelet count: 153×10^3/µl. Differential count was-blast: 02%, myelocytes: 03%, metamyelocytes: 14%, neutrophils: 39%, lymphocytes: 05% and monocytes were 36%. Bone marrow aspiration showed hypercellular marrow having increased proliferation of monocytic series with many mature monocytes, promonocytes and occasional monoblasts. Megakaryocytes were increased in number with abnormal maturation. Bone marrow differentials were- blast: 03%, metamyelocytes:48%, neutrophils:11%, monocytes: 37% and plasma cells: 03%. Immunophenotyping of bone marrow showed 0.5% myeloblast, 54% non-blast myeloid cells showing reduced side scatter with abnormal maturation and monocyte ($\sim$ 32%) showing abnormal pattern on CD13 vs CD11b, CD14 vs CD11b, HLA DR vs CD14 on dot plot and mild expression of CD56. Bone marrow biopsy findings were consistent with aspiration findings and reticulin stain showed grade II fibrosis. Monoclonal protein was detected on S. electrophoresis. IgG lambda and lambda monoclonal protein detected on immunofixation. USG findings suggested mild hepatosplenomegaly. Patient was medically unfit for bone marrow

transplant in view of age hence, counselled for other treatment plans. Unfortunately, patient succumbed to death imposing no further investigation.

Conclusion: CMML is a rare disease with a heterogeneous clinical presentation. Recent progresses in the molecular and cellular pathogenesis of CMML have stirred a renewed interest in this clinically heterogeneous disorder.

P210 BCR-ABL1 (%IS -38.418) CML with CMML (TET2 48.9%, SRSF2-32.5%): Is It the First Case Report of Coexisting CML P210 & CMML Clones?

Mona Vijayaran, Gurleen Oberoi, Sanjay Mishra

Introduction: p190 BCR-ABL1 in CML is often associated with monocytosis. In the case described here, monocytosis is associated with coexisting p210 BCR-ABL and CMML clones. Mutation analysis using next-generation sequence (NGS) in our case showed TET2 and SRSF2 mutations.

Aims & Objectives: A 75 year male was evaluated for monocytosis and thrombocytopenia. CBC showed Hb-11.8 g/dl, TLC-12,060/cmm, Monocytes-35%, Platelets-39,000/cmm.

Materials & Methods: Bone marrow examination showed a hypercellular marrow with myeloid series showing sequential maturation upto neutrophils with 30% monocytes. Immunophenotyping by flowcytometry from bone marrow had 3% blasts. Making chronic myelomonocytic leukemia as the likely diagnosis. NGS for myeloid mutation panel had TET2 (48.9%) and SRSF2 (32.5%) mutations. This report further supported the diagnosis of CMML. To fulfil the WHO diagnostic criteria for CMML, a BCR ABL1 by RQ-PCR was sent. The report came positive for P210 (B3A2, B2A2) Major Transcript (M-BCR) % IS of 38.418.

Result: The patient was counselled regarding the unique presentation of presence of 2 clones- P210 CML and CMML. After discussion with an international faculty with vast experience in CMML. It was decided to start this elderly gentleman on Imatinib 200 mg and not on azacytidine, as ASXL1 was not present, hence, his chances of progressing to AML would be less and on the other end, if CML is left untreated then chances of progression to blast phase would always be a possibility. After 3 months on Imatinib his platelet count improved to 80,000 to 90,000/cmm but his monocytosis persists. His 3rd month BCR-ABL1 IS% is 0.004%.

Conclusions: After searching the literature, there were no case reports of a coexisting CML p210 with CMML. This case might be the first case report. P190 BCR ABL1 is often associated with monocytosis. There are few case reports of p210 BCR ABL1 positivity in patients with monocytosis but none with coexisting CMML. This case highlights the need for extensively evaluating patients with monocytosis with next generation sequencing for myeloid mutation panel and BCR-ABL1 by RT-PCR to correctly diagnose and treat them.

A Rare Presentation Of CML With Non Hodgkin Lymphoma

Swati Singh, Nilesh Kumar, Nitish Kumar Patel

Introduction: The simultaneous diagnosis of myeloproliferative disorder and lymphoid malignancy in the same patient is extremely uncommon.Even if reported, the most common combination is philadelphia negative myeloproliferative neoplasm with chronic lymphocytic leukemia.The -occurence of BCR -ABL positive CML and NHL is rare.

Aims & Objectives: To present a case of synchronously occuring Chronic Myeloid Leukemia and Non Hodgkin Lymphoma (Diffuse large B cell lymphoma) in a 41 year old newly diagnosed CML patient.

Materials & Methods: Here we report the case of a 41 year old female patient who presented to us with the complains of generalised weakness and easy fatiguability and multiple swellings in the inguinal region. On physical examination, she has massive splenomegaly and generalised lymphadenopathy. A complete blood count showed severe anaemia and leukocyosis in the range of 3 lakhs/Cu mm.Peripheral blood picture, bone marrow examination and lymph node biopsy was done. General blood picture showed the picture suggestive of myeloproliferative disease with only few circulating blast cells.BCR ABL was positive and chromosomal analysis showed Philadelphia chromosome in 100% of cells.Similarly,bone marrow examination also showed hypercellular marrow,with trilineage hematopoiesis,eosinophils and megakaryocytic hyperplasia and only 3% blast cells,again suggestive of CML in chronic phase.Lymph node biopsy showed features suggestive of Non Hodgkin lymphoma,possibly Diffuse large B cell lymphoma.IHC was done which showed positivity for CD 79a,CD 19,CD 20. DD 10 and BCL6 were also positive.

Result: On the basis of a complex investigation-immunohistochemistry, conventional cytogenetic analysis,FISH and molecular analysis, the patient was diagnosed as Chronic myeloid leukemia in chronic phase with Diffuse Large B cell lymphoma.

Conclusions: A treatment naive CML patient presenting with lymphadenopathy on the first presentation is most commonly thought of as being in a blast crisis.However a slight degree of suspicion and careful further evaluation and investigations can prevent the erroneous diagnosis and aid in the much needed correct line of treatment as NHL needs an agressive plan of management.

Pleural Effusion As An Extramedullary Manifestation Of CML In Chronic Phase

Ankita Dewangan, K K Gupta, Nilesh Kumar, Jitendra Singh, Bhavya J Nair

Introduction: Figure 1: Pleural fluid cytology.

Patients with Chronic myeloid leukemia (CML) in chronic phase usually presented with features of anemia, and splenomegaly. Extramedullary disease is uncommon in chronic phase accounts only 2% patients, usually involving skin, subcutaneous tissue, bone, lymph-node and CNS. Involvement of pleura is very rare in chronic phase.

Aims & Objectives: To present case of unilateral pleural effusion as an extramedullary manifestation of CML in chronic phase in 48 year old male.

Materials & Methods: A 48 year old male, smoker presented with chief complaint of breathlessness and left sided chest pain since 1 month. It was associated with loss of appetite, weight loss and generalized malaise. On examination patient was tachypneic and had pallor. Respiratory system examination revealed reduced chest movements and air entry on left side. Abdominal examination did not reveal any liver or spleen enlargement.

Chest skiagram suggested left sided moderate pleural effusion. Pleural fluid analysis showed raised total cell count of 34,650/mm3 with differential count comprised of 37% neutrophils, 4% lymphocytes, 24% stab forms, 20% myelocytes, 15% metamyelocytes. A complete blood count showed anemia and marked leukocytosis of 1.2 lacs/mm3. Peripheral blood smear showed numerous premature

myeloid series comprised of myeloblasts 5%, myelocytes 17%, metamyelocytes 16%, neutrophils 47%, eosinophils 4% and basophils 9%, suggesting CML in chronic phase. Bone marrow aspiration revealed hypercellular bone marrow and numerous premature forms in myeloid series with 8% blast cells, suggesting CML in chronic phase. Chromosomal studies suggested presence of Philadelphia chromosome in 100.

Result: Based on clinical impression and investigation diagnosis of CML in chronic phase with left sided pleural infiltration was made suggesting extramedullary manifestation of disease.

Conclusions: Sole presentation of pleural effusion in chronic phase of CML is rare. Hematopoietic malignancy should be in differential diagnosis of unilateral pleural effusion. Awareness of this uncommon situation by both physician and cytopathologist is critical for the diagnosis and management of such cases.

Epidemiology And Risk Stratification As Per Eutos Long-Term Survival Score (ELTS) Of Patients With Newly Diagnosed Chronic Phase Chronic Myeloid Leukemia: Analysis From A Single-Center

Sashi Kant Singh, Karthik Kumar, Jhasketan Nayak, Jasmine Porwal, Gaurav Dhingra, Uttam Kumar Nath

Introduction: Risk stratification at the time of diagnosis is a key element for prognosis and management of chronic myeloid leukemia—chronic phase (CML- CP). The baseline EUTOS long-term survival score (ELTS) provides prognostic information about CML-related death and overall survival.However, the disease epidemiology and risk stratification scores are based mainly on data from western countries and there is paucity of data from Indian population.

Aims & Objectives: To study epidemiological characteristics and ELTS risk stratification among newly diagnosed CML CP patients presenting to AIIMS Rishikesh.

Materials & Methods: The study enrolled newly diagnosed CML-CP patients after obtaining informed consent.

Inclusion Criteria:

Newly diagnose CML-CP.

Age $\geq$ 12 years.

Exclusion Criteria:

de novo accelerated or blast phase CML.

The ELTS risk score was calculated at diagnosis.

Result: Total 119 consecutive CML patients were screened between April 2021 and August 2022 out of which 106 patients with confirmed diagnosis of CML in Chronic phase were enrolled in the study. The median age of patients was 34 years (inter-quartile range 19 years), with male: female ratio of 1.4: 1. The distribution of these patients as low risk, intermediate risk and high risk as per the ELTS risk stratification is 25.5% (n = 27), 42.5% (n = 45) and 32.1% (n = 34) respectively.

Conclusions: In this study, the median age at diagnosis of CML is 34 years which is much lower compared to studies reported from the western world where the median age is in the 6th decade of life and so is suggestive of onset of CML at a younger age in the Indian population.

Therapeutically, Tyrosine kinase inhibitors (TKIs) have been reported to provide a life expectancy similar to General population in the western world but whether the same success with TKI s can be achieved when the disease onset is in a younger population needs to be evaluated.

The study also shows that almost ¾ th of the patients fall in the intermediate and high risk ELTS group where 2nd generation TKIs may be more effective necessitating their easy accessibility and financially affordability.

Rare Presentation Of Synchronous Dual Malignancy (Mds/Mpn With Ring Sideroblasts And Thrombocytosis With Gastric Adenocarcinoma) In An Old Age Patient: New Horizons To Explore

Neha Mala Krishna, Ankita Kumari, Kanwaljeet Singh, P Sengupta, Devika Gupta, Priyanka Mishra, Rajan Kapoor

Introduction: The incidence of dual malignancies has increased over past years due to improved diagnostic modalities/therapeutics and their frequency ranges from 2–17% which may be synchronous or metachronous. The etio-pathogenesis may be multifactorial e.g., radiotherapy, environmental factor or may be genetic mutations etc. As per literature search, maximum prevalence of solid- solid dual malignancies have been reported and there is sparse data regarding dual malignancy combination of solid- hematological tumors.

Aims & Objectives: This case report highlights a rare presentation of MDS/MPN with ring sideroblasts & thrombocytosis with synchronous carcinoma stomach in a 62-year-old patient and this is first ever case of such combination as per literature search. This case report will be helpful in making clinicians aware of such presentation and further highlighting the importance of timely intervention and management planning in these patients.

Materials & Methods: We discuss a rare case, a 62-year female who came with a chief complain of abdominal pain, vomiting and anemia. During laboratory investigations, anemia with mild leucocytosis & thrombocytosis was found. Further spectrum of investigations finally led to diagnosis of gastric carcinoma in the background of MDS/MPN.

Result: Peripheral blood showed dimorphic anemia, neutrophilic leukocytosis and thrombocytosis. Bone marrow aspirate was haemodiluted and showed few erythroid & myeloid precursors with occasional dyspoietic megakaryocyte. Perl's stain revealed ring sideroblasts. Bone marrow biopsy showed metastatic deposit of adenocarcinoma (PAN-CK, CK7, CK20, CK19 positivity) with background showing hypercellularity of hematopoietic cells (predominantly megakaryocytic hyperplasia with dyspoietic hypolobated forms and myeloid bulge). Further investigations showed biopsy proven carcinoma stomach with similar IHC profile as in bone marrow. Molecular mutational analysis carried on bone marrow sample in private lab showed presence of JAK2V617F and SF3B1 mutation. Final diagnosis of dual synchronous MDS/MPN with ring sideroblasts & thrombocytosis with gastric carcinoma was made.

Conclusions: In conclusion, this case report is presented to highlight such unique rare synchronous dual malignancies so that clinicians are aware of such presentations and have high index of suspicion which can lead to detailed evaluation of the patient and will open new horizons in diagnostics and management of dual malignancies.

Myeloproliferative Neoplasm (MPN) With Chronic Myelomonocytic Leukaemia (CMML) Like Phenotype -2 Interesting Cases With Monocytosis

Pradeep Arumugam, Suneet Kaur Hora

Introduction: MPNs with monocytosis has been reported as a part of rapid progression of the disease and with persistent peripheral blood monocytosis which is a hallmark of the patients with CMML. Such a CMML-like phenotype can be seen in patients with MPN. These two cases which had the diagnostic dilemma of CMML in morphology was diagnosed as myeloproliferative neoplasm.

Aims & Objectives: To highlight the fact that monocytosis in a suspected case of MPN has a rapid progression in the course of the disease mimicking CMML like phenotype.

Materials & Methods: Case 1: 83 year old male presented with fatigue followed up as myeloproliferative neoplasm with a suspicion

of CMML as possible diagnosis. He had an initial counts of platelets 12 lakhs MPL mutation was detected and all others were negative. One year of follow up he had absolute monocytosis which persisted. Case2:72 year old male presented with anemia who was admitted and evaluated for MPN after a month with a suspicion of MPN as patient presented with leukocytosis and monocytosis. Reflex panel detected JAK2V617F mutation.Throughout the 6 months he had absolute monocytosis persistent more than 3 months.

Result: Monocytosis is a hematologic feature that can be found in several reactive and clonal conditions. Both the cases had a history with a diagnosis of MPN and both had an initial suspicion of CMML as there was persistent and absolute monocytosis. But however with complete clinical morphological and molecular profiles, recognition of this monocytosis as an indicator of rapid progression of the disease was identified.

Conclusions: Development of monocytosis in patients with established primary myelofibrosis is associated with rapid disease progression and these patients should be considered as a high-risk group associated with short survival.

Survey of Additional Chromosomal Abnormalities at Diagnosis in Chronic Myeloid Leukemia in a Regional Genetics Laboratory

Shivani U, D Prashanth Shetty, Reshma Shetty, Vijith Shetty, Gloria Venissa Quadros, Dr. Rajesh Krishna

Introduction: Chronic Myeloid Leukemia (CML) is a myeloproliferative neoplasm, characterized by the presence of a translocation between chromosomes 9 and 22 leading to the formation of the Philadelphia(Ph) chromosome. The emergence of additional chromosomal abnormalities (ACAs) in Ph positive chromosome, is seen in disease evolution. However chromosomal aberrations may be noted at diagnosis.

Aims & Objectives: To evaluate additional chromosomal abnormalities in CML.

Materials & Methods: The study was conducted between 2017 to 2021 in collaboration with 2 tertiary hospitals of coastal Karnataka. Conventional Karyotyping was carried out on bone marrow or peripheral blood using Giemsa banding technique. Analysis was done using GenASIs software version 8.1. Results were interpreted according to International System of Human Cytogenetic Nomenclature. Fluorescent In Situ Hybridisation (FISH) analysis was carried out on interphase and metaphases. FISH probes were used to detect BCR/ABL1 fusion. Haematological and clinical parameters were recorded.

Result: The study comprised of 84 CML Ph positive patients. Only 6 cases had additional chromosomal abnormality. One case showed double Ph and probe hybridization confirmed the presence Ph chromosome in 96% of the cells analysed. Another case with double Ph with isochromosome 17q, revealed varied signal pattern (3F,1R,1G; 2F,1R,1G). A case with three-way translocation (9;22;21) showed 1 fusion on der (22) 1 green on normal 22 and 1 more green on chromosome 21 and 2 Red signal on chromosome 9. A case with gain of X chromosome confirmed abnormal signal pattern. 1 case had normal female karyotype 46, XX, whereas FISH confirmed positive. 1 case revealed 46, XY, t(9;22) with few random missing and marker chromosome. FISH results confirmed 40% of cells suspected for three- or four-way translocation (2R + 2G + 1F) remaining cells with varied signal pattern. All the 6 cases were asymptomatic, however had leukocytosis and splenomegaly.

Conclusions: Our study of 84 consecutive CML cases over 5 years showed that additional genetic aberration may be seen at diagnosis, though it is a rare occurrence (6/84), The long term prognostic implication of these findings are under study and a multi centre long term study in India would immensely help in understanding the biology, prognostic implications and treatment options.

Chronic Myeloid Leukemia With Lymphoblastic Crisis In Childhood: An Unusual Case

Vaanya Kaushik, Sushma Belurkar

Introduction: Chronic Myeloid Leukemia (CML) accounts for less than 3% of newly diagnosed leukemia in children. Pediatric CML has a similar natural history and biology to adult CML and follows a triphasic pattern. Blast crisis (> 20% blasts in the marrow or the presence of extra-medullary blast proliferation) is even more unusual in pediatric age group with lymphoblastic crisis being infrequent compared to myeloblastic crisis.

Aims & Objectives: We report a case of a child who presented to our hospital, with the disease.

Materials & Methods: A 5-year-old boy presented to the hospital with one-month history of intermittent fever and ecchymotic patches all over the body. On examination, he was found to have significant splenomegaly and bilateral cervical lymphadenopathy.

Result: His complete blood count showed a hemoglobin level of 7.3 g/dL, a platelet count of 28 × 109/L, and a total white cell count of 365 × 103 u/L. A differential count showed the following: blasts, 36%; promyelocytes, 3%; myelocytes, 10%; metamyelocytes, 3%; neutrophils, 28%; eosinophils, 2%; basophils, 1%; and lymphocytes, 8%.

Bone marrow aspirate showed myeloid hyperplasia with 32% blasts.

Flow cytometry analysis showed blasts expressing CD19, CD10, cCD79a, CD20, HLA-DR, CD34, and cMPO. Since all other myeloid antigens were negative, it was not diagnosed as Mixed Phenotypic Acute Leukemia (MPAL) as the score did not satisfy the European Group of Immunological Classification of Leukemia (EGIL) criteria.

FISH revealed BCR-ABL1 fusion: t(9;22)(q34;q11) translocation.

Correlating clinical symptoms, bone marrow findings, and molecular tests resulted in a final diagnosis of CML in B-cell lymphoid blast crisis.

However, the child had CNS bleed on 5th day of admission and succumbed even before the treatment could be started.

Conclusions: In the absence of a documented CML chronic phase, distinguishing between lymphoid blast crisis of CML and a Philadelphia chromosome-positive ALL can be challenging. Hence a comprehensive approach including clinical and laboratory data is required for an early diagnosis and effective management.

Validation Of Bcr-Abl Immunohistochemistry In Bcr-Abl Positive Leukemia At Presentation And In Follow-Up Cases On Bone Marrow Biopsy

Balaji K, Ashwani Tandon, Shrirang Deepak Pathak,Sachin Bansal

Introduction: Chronic Myeloid Leukemia (CML) is characterized by fusion of the BCR and ABL1 genes caused by the t(9,22) translocation (q34,q11). The current CML diagnosis relies on either karyotyping for the Philadelphia(Ph) chromosome or FISH/RT-PCR for the BCR-ABL1 fusion oncogene. The resultant BCR-ABL1 protein produced by this oncogene is unexplored for diagnostics. The antibody against this protein is available for research purposes and needs validation by immunohistochemistry(IHC) as a diagnostic tool.

Aims & Objectives: To standardize the immunohistochemistry-based diagnostics of BCR-ABL1 oncoprotein with Phospho-BCR antibody in BCR-positive leukemia.

Materials & Methods: This is a retrospective pilot study conducted at the Department of Pathology, AIIMS, Bhopal. Formalin-fixed paraffin-embedded bone marrow biopsies diagnosed as BCR-ABL

positive leukemia fulfilling the pre-set inclusion and exclusion criteria were retrieved from the department archive. A total of 35 biopsies were included in the current study, comprising 26 biopsies of CML, 4 Philadelphia positive Acute Lymphoblastic Leukemia (Ph + ve ALL) and 3 myeloproliferative neoplasms. Two of the Ph + ve ALL cases were assessed with both at-diagnosis and day-35 remission biopsies. These blocks were subjected to immunohistochemistry with Phospho-BCR (Tyr177) Polyclonal Antibody (Product # PA5-17,708) at 1:200 concentration after overnight incubation.

Result: Out of the 26 CML biopsies included, 25 cases showed moderate to strong membranous and cytoplasmic positivity in around 90% of the cells and 95 to 100% of the megakaryocytes showed strong cytoplasmic staining with a sensitivity of 96%. All four Ph + ve ALL cases at-diagnosis showed strong membranous and cytoplasmic positivity in 90% of cells and the two follow-up cases at day-35 remission showed moderate cytoplasmic positivity in 60% of cells. Both at-diagnosis and day-35 remission marrows of Ph + ve ALL showed positivity but the later had lesser intensity and lacked membranous staining. All three non-CML myeloproliferative neoplasms showed faint non-specific staining.

Conclusions: Our study suggested that BCR-antibody can be used as a reliable diagnostic marker in both CML and Ph + ve ALL cases. The preliminary results of BCR antibody are encouraging and needs to be further validated with a cocktail of both BCR and ABL component which might give result with near concordance to cytogenetic and molecular marker level of sensitivity in BCR-positive leukemia diagnosis.

Limitations: Cases of CML in remission are not included in this pilot study.

Diagnosis	Number of cases	Number of cases showing immunoreactivity	Number of cases showing no immunoreactivity	Percentage of cells involved	Site
CML-CP	24	23	1	80% to 90% Strong positivity	Membranous and cytoplasmic
CML- Blast crisis	1	1	0	90% strong cytoplasmic	Membranous and cytoplasmic
CML non responsive to treatment	1	1	0	70% Moderate	Cytoplasmic
Ph positive ALL at diagnosis	4	4	0	90% Strong positivity	Membranous and cytoplasmic
Ph positive ALL – at remission	2	2	0	60% moderate positivity	Cytoplasmic
Myeloproliferative neoplasms	3	3	0	Faint staining	Non-specific

Table depicting the distribution of BCR-ABL positive leukemia and percentage of cells showing immunoreactivity and and site of staining

A Rare Case Of Smouldering Systemic Mastocytosis In A 4 Year Old- A Case Report

Shinjini Choudhury, Shailaja Shukla, P L Jyotsna,Sharmila B Mukherjee

Introduction: Mastocytosis is a heterogenous group of disorders that is characterized by excessive proliferation and pathologic accumulation of mast cells in various body tissues. The mast cells also have abnormal morphology and aberrant expression of surface receptors. Mastocytosis, in all its forms, is a rare disorder, with an estimated prevalence at 1 per 10,000 persons. Systemic mastocytosis is rarely seen in children, while Smouldering Systemic Mastocytosis characterized by high mast cell burden and organomegaly, is even rarer.

Case Report: A 4-year-old boy presented with generalized skin lesions since birth and abdominal distension for 3 years.

Examination findings revealed severe acute malnutrition, pallor, facial hypertrichosis, polymorphous skin lesions (cicatricial alopecia, diffuse erythema, multiple plaques of variable diameter, skin-colored nodules and hypertrophic irregular scars with a positive Dariers sign suggestive of blistering), hepatosplenomegaly and generalized lymphadenopathy.

Haemogram was suggestive of macrocytic anemia and bicytopenia. Serum vitamin B12 level was low. Serum tryptase level was increased (> 200 μg/L).

Fine needle aspiration cytology (FNAC) of an inguinal lymph node showed polymorphous lymphocytes with numerous mast cells. Skin biopsy taken from a nodule displayed positive special staining for mast cells. Bone marrow aspirate showed an increase in mast cells in clusters, as well as scattered spindle forms.. Bone marrow biopsy (BMB) revealed hypercellular marrow spaces (approximately 100%) with infiltration by multifocal compact clusters of atypical mast cells which were positive for CD 117 on immuno-histochemistry.

Based on the clinical features and WHO 2016 diagnostic criteria, for systemic mastocytosis and its variants, a diagnosis of Smouldering Systemic Mastocytosis with vitamin B12 deficiency was made.

The patient was managed for Severe Acute Malnutrition and Nutritional Deficiencies, while cutaneous manifestations were treated with oral hydroxyzine, levocetrizine, topical tacrolimus and calamine lotion.

Conclusions: In this case, the patient showed the presence of recurrent blisters since birth, which is an exceptionally rare presentation and was initially misdiagnosed as a bullous disorder. As there is currently no available cure, the goals of treatment are to mitigate organ damage, alleviate symptoms, improve the quality of life, and achieve long-term disease control.

A Rare Case Of Of Hyperbasophilia: "Acute/Chronic Basophilic Leukemia Vs Chronic Myeloid Leukemia In Accelerated Phase- A Diagnostic Dilemma

Rama Hariharan, Jasmita Dass, Ganesh Kumar V, Mukul Aggarwal, Rishi Dhawan, Tulika Seth, M Mahapatra

Introduction: Chronic basophilic leukemia is a rare entity with few only few cases reported so far. In this article we present a case of a young female with a short duration of symptoms with peripheral blood showing blasts and basophilia. Bone marrow was hypercellular with presence of blasts and abnormal basophils including eosinophil-basophil precursors posing a diagnostic challenge.

Aims & Objectives: To report a rare case of chronic basophilic leukemia associated with BCR-ABL1 and to revisit the proposed diagnostic criteria of basophilic leukemias.

Materials & Methods: 32 year old woman presented with complaints of fever with bruises all over the body and quantified weight loss of 5 kgs. Investigations: Hb-6.1gm/dl, TLC- 6640/μl, Platelet count-33,000/μl. Peripheral smear showed 7% blasts and basophilia(58%). Bone marrow was hypercellular with prominence of basophils and dyspoietic myeloid precursors. Flow cytometry revealed the following immunophenotype- CD123 + , CD33 + , CD38 + , CD25 (dim) and negative for CD117, HLA DR, CD34, MPO in these cells. RT-PCR for BCR-ABL1 showed P210 transcript. Cytogenetic analysis showed multiple chromosomal abnormalities in addition to t(9:22)

Result: Based on morphology, bone marrow findings and cytogenetics a diagnosis of CML-AP vs Chronic basophilic leukemia secondary to CML was given. She was started on Dasatinib100mg OD and was given transfusion support Follow up after 3 months of treatment revealed improved CBC parameters with no blasts and basophils on peripheral smear.

Conclusions: World Health Organization has included acute basophilic leukemia (ABL) as a distinct entity in the classification of hematologic malignancies. No generally accepted criteria for the diagnosis and classification of basophilic leukemias have been generated. Marked basophilia is usually seen in the presence of an

underlying hematologic malignancy, such as a myeloproliferative neoplasm (MPN), acute myeloid leukemia (AML), or acute lymphoblastic leukemia (ALL). Chronic myelogenous leukemia (CML) is one of the most common types associated with basophilia. Classification of basophil transformation in myeloid neoplasms and basophilic leukemias with defined criteria may lead to a commonly used nomenclature and would support harmonizing research and assist in daily practice.

Disease variant	Proposed criteria (Valent et al)
ABL	Myeloblasts + metachromatic blasts ⩾20% and basophils ⩾40% of nucleated BM or PB cells (+HB criteria fulfilled)
Primary ABL	No preceding or underlying BM neoplasm
Secondary ABL	Known preceding/underlying BM neoplasm
CBL	Myeloblasts + metachromatic blasts <20% and basophils ⩾40% of nucleated BM or PB cells (+ HB criteria fulfilled)
Primary CBL	No preceding or underlying BM neoplasm
Secondary CBL	Known preceding/underlying BM neoplasm

Early T Precursor Lymphoid Blast Crisis In Chronic Myeloid Leukemia-An Extremely Rare Presentation With Review

Chinmayee Panigrahi, Gaurav Chhabra, Nakul Tikare, Prapti Acharya, Somanath Padhi, Prabodha Kumar Das

Introduction: Chronic myeloid leukemia (CML) is one of the most common myeloproliferative neoplasm with an overall incidence of 1–2 cases per 100,000/year and resulting from a reciprocal translocation t(9:22) leading to formation of a fusion gene product. The disease course is triphasic with an initial indolent chronic phase followed by accelerated phase and blast crisis (BC)- that may be usually lymphoid or may be myeloid. A T-cell lymphoblastic crisis has been very rarely described in CML.
Aims & Objectives: We describe a rare case of CML presenting with Early T cell precursor (ETP) lymphoid BC in an adult male.
Materials & Methods: A 30-year- old male presented with history of loss of appetite for three months with significant weight loss. He complained of worsening fatigue and dyspnea on exertion. Physical examination revealed splenomegaly. Peripheral smear revealed leukocytosis with left shift, basophilia, with 6% blasts. The bone marrow aspirate & biopsy showed increased cellularity with suppressed erythropoiesis with normal and orderly maturation. Myelopoiesis was increased with 3% blasts and 10.
Result: Lymphoid BC constitutes the predominant phenotype of blast transformation in CML. The cases with lymphoid BC in CML are predominantly B cell type with very few cases of T cell phenotypes reported till date.
Conclusions: Careful flow cytometric evaluation with a larger set of panels is warranted for diagnosis of ETP-phenotype as ETP ALL is associated with poor outcome and owing to the rarity of its association with CML blast crisis, little is known about the clinical behavior and response of these patients to the traditional therapeutic regimens.

Molecular Characterization Of Juvenile Myelomonocytic Leukemia (JMML): Case Series From A Tertiary Care Center

Sathya Mani, Madhavi Maddali, Uday Prakash Kulkarni, Fouzia N A, Sharon Anbumalar Lionel, Sushil Selvarajan, Biju George, Vikram Mathews, Aby Abraham, Poonkuzhali Balasubramanian

Introduction: Juvenile myelomonocytic leukemia (JMML) is a rare, aggressive childhood myeloproliferative neoplasm that manifests as increased infiltration of the peripheral blood, bone marrow, and viscera by abnormal myelomonocytic cells. JMML diagnosis is made by combining clinical, laboratory, and molecular criteria. Most patients with JMML have somatic and/or germline mutations of genes within the RAS/MAPK signaling pathway.
Aims & Objectives: This study aimed to evaluate the genomic profile in JMML.
Materials & Methods: This retrospective study included all cases suspected of JMML based on clinical and pathological findings and sent for further evaluation by cytogenetics and molecular assays between Jan 2020 to August 2022. Karyotyping, FISH, and RTPCR were performed according to standard protocols. Targeted next-generation sequencing (NGS) was performed using a customized gene panel on the Illumina platform at a read depth of $\geq$ 250x. The data were analyzed using standard bioinformatic pipelines. Variant annotation and classification were done according to AMP/ACMG guidelines.
Result: The median age of the 6 cases (4 males and 2 females) included in this study was 3 years (Interquartile range: 1 to 4 years). Five patients had normal karyotypes, and one had t(8;21). All patients were negative for BCR::ABL1. PTPN11 mutation was detected in (5 out of 6 cases) and one case had NRAS mutation. Mutations in NF1 and CSF3R co-occurred with PTPN11 in 1 case each. All mutations in the PTPN11 and NRAS had a mutant allele burden of $\sim$ 50%.
Conclusions: Our study highlights that comprehensive genomic profiling identifies at least one mutation in all JMML patients. Performing genomic analysis at baseline might help triage children with JMML for allogeneic stem cell transplant in resource-constrained settings.

Juvenile Myelomonocytic Leukemia In The Setting Of PTPN11 Mutation And Noonan Syndrome: Report Of A Rare Case

Neha, Sunita Sharma, Kusha Sharma, Shailaja Shukla, Saumya Tiwari, Garima Chaudhary, Ashna Kumar

Introduction: Noonan syndrome is an uncommon genetic disorder belonging to group of genetic syndromes arising from germline mutations in the RAS/MAPK pathway. Hematologically, these children have a predisposition to develop Juvenile myelomonocytic leukemia (JMML) or JMML- like myeloproliferative disorders. The PTPN11 mutation in Noonan syndrome with JMML is known to have a favourable prognosis compared to sporadic JMML. We report a case of Noonan syndrome with JMML associated with heterozygous PTPN11 mutation and a rare ETV6—RUNX1 mutation. This unique association of JMML with PTPN11 and ETV6—RUNX1 mutation is not well elucidated in literature.
Aims & Objectives: To study the clinicohematological and molecular findings consistent with JMML in a case of Noonan syndrome.
Materials & Methods: A 17 month old male child presented with high grade fever, severe anemia and massive hepatosplenomgaly. He also had maculopapular rashes on the face and horizontal nystagmus in both eyes. Clinical diagnosis of ? leukemia, ? sepsis and ? hemophagocytic lymphohistiocytosis (HLH) were considered.
Result: Hemogram revealed anemia (Hb 9.5 g/dl), leucocytosis (TLC 30 $\times$ 103/µl) and thrombocytopenia (43 $\times$ 103/µl). Peripheral smear showed leucoerythroblastic blood picture with occasional blasts and absolute monocytosis (1500/µL). Bone marrow aspirate smears showed erythroid hyperplasia with normoblastic maturation, with few dyserythropoietic cells. Myeloid series showed normal maturation with presence of few atypical cells (3%) having high N:C ratio, scant cytoplasm, round nuclei, dispersed chromatin and occasional nucleoli. His serum uric acid levels were elevated (9.1 mg/dl). High

Performance Liquid Chromatography showed high HbF (47%). Blood and urine cultures were sterile. HLH investigations were negative. Based on these results, a possibility of JMML was considered. Whole exome sequencing revealed heterozygous PTPN11 mutation in exon 13 c. 1520G > T (p.Gly507V) suggestive of Noonan syndrome. Bcr-abl mutation was negative however, a unique ETV6 RUNX1 mutation was found. Thus, a final diagnosis of Noonan syndrome with Juvenile Myelomonocytic Leukemia was made. The child is undergoing chemotherapy and is presently asymptomatic.

Conclusions: Noonan syndrome associated with JMML is an uncommon entity which may result in misdiagnosis, delayed treatment and life threatening hematological complications. Hence, it is essential to adapt a holistic approach including clinical, hematological and molecular characteristics to arrive at correct diagnosis.

Asciminib In Chronic Myeloid Leukemia- An Initial Experience Of Stamp Inhibitor From A Tertiary Cancer Center In India

Rajat Pincha, Arijit Nag, Jeevan kumar, Saurabh Jayant Bhave, Vivek Radhakrishnan, Rizwan Javed, Manik Ghosh, Reena Nair, Mammen Chandy

Introduction: Asciminib, a first in class allosteric inhibitor targeting the ABL myristoyl pocket was approved by US-FDA for CML-CP with resistance or intolerance to 2 prior lines of therapy including patients with T315I mutation.

Aims & Objectives: To describe the initial experience with the use of Asciminib in an Indian setting with focus on tolerability and safety.

Materials & Methods: A retrospective chart review of 6 adult CML-CP patients who received asciminib at our center was done. Patients either had T315I mutation or were resistant/intolerant to prior TKI. Starting dose was 200 mg BD and 80 mg BD for T315I and non-T315I mutation respectively. Dose reduction/discontinuation was done as per published guidelines. Toxicities were graded as per CTCAE version 5.

Result: Baseline characteristics are illustrated in table 1. All patients were male with a median age of 45 years. Median duration of therapy was 182 days (range: 79–350). Response at 3 months was available in 4 patients, none of them achieved BCR::ABL < 10%, one patient progressed to accelerated phase and therapy was discontinued. At 6 months, one patient progressed (increase in BCR::ABL levels), while none achieved optimal response (BCR::ABL < 1%). However four patients remained in hematological remission. Adverse events noted were ≥ grade 3 hematological in two patients and one patient developed pancreatitis. Of the patients intolerant to prior TKI due to hematological toxicity, (n = 6), four could tolerate the drug. Asciminib was discontinued in 5 patients (failure of response in 3 patients, pancreatitis in one patient and grade 4 hematological toxicity in one patient). One patient is continuing on therapy.

Conclusions: Asciminib is a promising agent in CML-CP which can be safely delivered in most patients. We would need more experience to comment on efficacy in our cohort.

(*We would like to acknowledge Novartis for their support in providing Asciminib for companionate usage for our patients*).

Table 1: Baseline Characteristics of patients.

Particulars	Number of patients
Total	6
Median Age in years (range)	45 (25-74)
Sex	
Male	6
Female	0
Stage of disease	
Chronic phase	6
Tyrosine Kinase Domain mutation	
None	3
T315I	3
Median Prior line of therapy (range)	3 (1-3)
Starting Dose	
80mg BD	3
200mg BD(T315I)	3

Efficacy And Safety Of Ponatinib In Chronic Phase CML- Real World Data From A Tertiary Care Centre In Eastern India

Dibakar Podder, Arijit Nag, Jeevan Kumar, Saurabh Bhave, Vivek Radhakrishnan, Reena Nair, Mammen Chandy

Introduction: Ponatinib is a third generation tyrosine kinase inhibitor (TKI) currently approved for the treatment of adult patients with chronic phase, accelerated phase, or blast phase chronic myeloid leukemia (CML) that is resistant or intolerant to prior tyrosine kinase inhibitor therapy or Philadelphia chromosome positive acute lymphoblastic leukemia (Ph + ALL) that is resistant or intolerant to prior tyrosine kinase inhibitor therapy. However, ponatinib's safety data revealed a dose-dependent, increased risk of serious cardiovascular (CV) events.

Aims & Objectives: To describe the initial experience with the use of Ponatinib with focus on tolerability and safety.

Materials & Methods: A retrospective review of 16 adult CML- CP (chronic phase) patients with either T315I mutation or resistant/intolerant to prior TKI who were started on Ponatinib was done. Ponatinib was started at a dose of 15 mg once daily and gradually increased to 45 mg. Dose adjustments/discontinuation was done as per published guidelines. Toxicities were graded as per CTCAE version 5.

Result: Baseline characteristics are illustrated in table 1. 11 patients (68.8%) were males and 5 patients (31.3%) were females with a median age of 49.4 years. Median duration of therapy was 197 days (range: 15–730 days). RQ-PCR data was available in 10 patients. Optimal response (bcr:abl < 10% at 3 months or bcr-abl < 1% at 6 months or bcr-abl > 0.1% at 1 year) was seen in 4 patients. Suboptimal responses were seen in 6 patients. In 3 patients ponatinib was discontinued due to untoward side effect. The remaining 3 patient has not completed 3 months of therapy. Grade ¾ anemia was seen in 4 cases (25%); Grade ¾ neutropenia was seen in 3 cases (18.8%) and Grade ¾ thrombocytopenia was seen in 5 cases (31.3%). 4 patients (25%) developed skin dryness and wrinkling. 2 patients developed elevation in transaminases and 1 patient had amylase elevation but with no clinical or imaging feature of pancreatitis.

Conclusions: Ponatinib is generally a highly effective drug and a relatively safe drug in a cohort of heavily pre-treated patients or patient with CML-CP with T315I mutation.

Table 1 Baseline Characteristics of patients

Particulars	Number of patients
Total	16
Median Age in years (range)	49.5 (23–75)
Sex	
Male	11
Female	5
Stage of disease	
Chronic phase	16
Tyrosine Kinase Domain mutation	
None	5
T315I	11
Median Prior line of therapy (range)	2 (1–4)

Efficacy and Safety of Ponatinib in Chronic Phase CML- Real World Data from a Tertiary Care Centre in Eastern India

Introduction: Ponatinib is a third generation tyrosine kinase inhibitor (TKI) currently approved for the treatment of adult patients with chronic phase, accelerated phase, or blast phase chronic myeloid leukemia (CML) that is resistant or intolerant to prior tyrosine kinase inhibitor therapy or Philadelphia chromosome positive acute lymphoblastic leukemia (Ph + ALL) that is resistant or intolerant to prior tyrosine kinase inhibitor therapy. However, ponatinib's safety data revealed a dose-dependent, increased risk of serious cardiovascular (CV) events.
Aim: To describe the initial experience with the use of Ponatinib with focus on tolerability and safety.
Methods: A retrospective review of 16 adult CML- CP (chronic phase) patients with either T315I mutation or resistant/intolerant to prior TKI who were started on Ponatinib was done. Ponatinib was started at a dose of 15 mg once daily and gradually increased to 45 mg. Dose adjustments/discontinuation was done as per published guidelines. Toxicities were graded as per CTCAE version 5.
Results: Baseline characteristics are illustrated in Table 1. 11 patients (68.8%) were males and 5 patients (31.3%) were females with a median age of 49.4 years. Median duration of therapy was 197 days (range: 15–730 days).RQ-PCR data was available in 10 patients. Optimal response (bcr:abl $< 10\%$ at 3 months or bcr-abl $< 1\%$ at 6 months or bcr-abl $> 0.1\%$ at 1 year) was seen in 4 patients. Suboptimal responses were seen in 6 patients. In 3 patients ponatinib was discontinued due to untoward side effect. The remaining 3 patient has not completed 3 months of therapy. Grade ¾ anemia was seen in 4 cases (25%); Grade ¾ neutropenia was seen in 3 cases (18.8%) and Grade ¾ thrombocytopenia was seen in 5 cases (31.3%). 4 patients (25%) developed skin dryness, xerosis and wrinkling. 2 patients developed elevation in transaminases and 1 patient had amylase elevation but with no clinical or imaging feature of pancreatitis.
Conclusion: Ponatinib is generally a highly effective drug and a relatively safe drug in a cohort of heavily pre-treated patients or patient with CML-CP with T315I mutation.
(*We would like to acknowledge Novartis and Takeda for their support in providing Ponatinib for companionate usage for our patients*).

Kikuchi-Fujimoto Disease: Misdiagnosed As Malignant Lymphoma Or Tuberculosis

Priyam, Namrata Sinha, Avinash Kumar

Introduction: Kfd is a rare and benign cause of lymphadenopathy. Due to its non specific presentation it is often misdiagnosed. Febrile lymphadenopathy not responding to first line antibiotics is either primarily diagnosed as extra pulmonary tuberculosis or because of its clinical semblance 1/3 KFD is misdiagnosed as malignant lymphoma.
Aims & Objectives: We present to cases of KFD both misdiagnosed as tb and lymphoma respectively. The aim of presentation is to increase awareness abd shed light on typical and atypical presentations of KFD to reduce the incidence of misdiagnosis.
Materials & Methods: Study design: case report.
Study instiitute: paras hmri, bailey road, patna.
Study tool: histopathology and laboratory investigations.
Result: We present 2 cases of a 19 and 10 year old male, both presented with febrile lymphadenopathy, leucopenia, increased esr and ldh. Histpathological examination of excised lymph nodes demonstraed crescentric histiocytes with karyorrhexis in a background of necrosis. Neutophil and granuloma were absent.afb was absent and no fungal element. Ihc was done to rule out other lymphoproliferative disorders.
Conclusions: It is importantto raise awareness of kfd, a benign and self limiting condition with good prognosis which has clinical presentation mimicking grave conditions like extra pulmonary tb and lymphoma. Timely histo pathological analysis can help avoid anxiety surrounding a misdiagnosis and its unnecessary treatment.

Role Of Imatinib Plus Thalidomide In Imatinib Failure Chronic Myeloid Leukemia: A Follow Up Data Of Six Months

Moupali Ghosh, Siddhartha Sankar Ray

Introduction: Imatinib failure is a well known problem in treatment of Chronic myeloid leukemia. Hence, more effective treatment strategies including newer generation tyrosine kinase inhibitors, different drug combinations are underway for improvement of outcome in Imatinib failure CML patients.
Aims & Objectives: The purpose of this study is to provide a cheaper alternative to newer generation Tyrosine kinase inhibitors in a resource constraint setting with combination therapy of Imatinib plus Thalidomide in Imatinib resistant CML cases, to determine the response rates and to look for its side effect profile.
Materials & Methods: The study was conducted on Imatinib resistant Cml patients with negative Tyrosine Kinase domain mutation attending CML clinic of Institute of Hematology and Transfusion Medicine, Kolkata. Patients were started on combination therapy of Imatinib and thalidomide after taking proper informed consent. Follow up was done from March 2022 to August 2022 with complete blood count, hepatic,renal function test every month and Bcr Abl every 3 months.
Result: Twenty Imatinib failure CML patients were followed up for 6 months; 14 males and 6 females treated of Imatinib (600 mg) and thalidomide (100 mg) as maximum tolerated dose. The time to follow up was 6 months. The age range was 13–65 years. Complete hematological response was achieved in 90% (18/25) of patients at 3 months, 5% (1/20) progressed to blast crisis. There was complete cytogenetic response in 70% (14/20). Major molecular response in 25% (5/20). MR 4.0 was achieved in 20%(4/20), 0.05% (1/20) had MR 5.0. There is no loss of response in those 18 patients on follow up. Two out of eigteen patients needed Imatinib dose reduction to 400 mg and 14 patients had Thalidomide dose reduction to 50 mg in view of side effect profile. Constipation, sleep disturbances and cytopenia were the most common side effects encountered.

Conclusions: The combination use of Thalidomide with Imatinib may provide promising results in Imatinib resistant CML patients. Further follow up studies are needed on a large population and for longer duration for evaluation of effectiveness of the combination therapy.

Sequential Diagnosis Of Follicular Lymphoma And Chronic Myeloid Leukemia In The Same Patient

Satarupa Mohapatra, Ashutosh Panigrahi, Somnath Padhi

Introduction: A 53 year old male presented in 2018, with gradually increasing swelling in the inguinal region for 6 months. On examination he had generalized lymphadenopathy and was diagnosed to have stage IV follicular lymphoma. After routine staging and pre-treatment evaluation, he was planned for 6 cycles of rituximab, cyclophosphamide, vincristine and prednisolone based therapy (RCHOP). Positron emission tomography- computerized tomography (PET-CT) done after 4 cycles of RCHOP was suggestive of complete metabolic response. Post 6 cycles of RCHOP, in view of the bulky abdominal disease, he was also planned for involved site radiotherapy (ISRT). After completion of 6 cycles of RCHOP and ISRT, repeat PET-CT also indicated complete response. Subsequently, he was considered for rituximab maintenance therapy, 3 monthly. However, since February 2020, he had palpable splenomegaly with progressively increasing total leucocyte count with left shift and basophilia. Initially for this he was put on observation. Nevertheless, his total leucocyte count continued to increase and splenic size increased as well. BCR- ABL RTPCR was positive for p210 transcript. He was then diagnosed to have chronic myeloid leukemia- chronic phase (CML-CP) and started on Imatinib. At the end of 12 months of treatment he had attained major molecular response. Patient continues to be on regular follow up. Diagnosis of both myeloid and lymphoid neoplasm in the same patient is usually very rare. Most common being association of a myeloproliferative neoplasm with chronic lymphocytic leukemia. Concurrent or sequential association of follicular lymphoma with CML is rare. Existing case reports have described either concurrent CML with follicular lymphoma or follicular lymphoma following CML. This is probably the first case report of sequential follicular lymphoma followed by development of CML.
Aims & Objectives: NA.
Materials & Methods: NA.
Result: NA.
Conclusions: NA.

Early Treatment Response As Per Eutos Long-Term Survival Score (ELTS) Risk Stratification In Newly Diagnosed Chronic Phase Chronic Myeloid Leukemia Patients: Analysis From A Single-Center

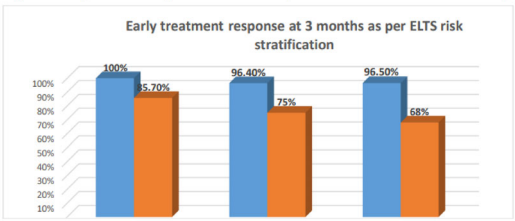
Sashi Kant Singh, Karthik Kumar,Jhasketan Nayak,Jasmine Porwal,Subhajit Hajra,B Priyavadhana,Gaurav Dhingra,Uttam Kumar Nath

Introduction: The recently validated EUTOS long-term survival score (ELTS) is the most useful predictor of CML-related death and CML-specific overall survival in chronic phase CML (CML-CP) and is recommended prior to initiation of tyrosine kinase inhibitor (TKI) therapy. There is paucity of data on early treatment response as per ELTS risk groups in Indian CML-CP patients.
Aims & Objectives: To study complete hematological response (CHR) & early molecular response (EMR) [defined as BCR-ABL1 transcript level $\leq$ 10%, I.S.] rates at 3 months of TKI therapy as per ELTS risk stratification among newly diagnosed CML-CP patients in a tertiary care hospital.
Materials & Methods: The study enrolled newly diagnosed CML-CP patients of age $\geq$ 12 years after obtaining informed consent. Patients with de novo accelerated or blast phase CML, and pregnant & lactating female patients were excluded. ELTS risk stratification was done at baseline and CHR & EMR rates at 3 months of therapy were analyzed.
Result: Total 119 consecutive CML patients were screened between April 2021 & July 2022, out of which 106 patients with confirmed diagnosis of CML-CP were enrolled. The median age of patients was 34 years (inter-quartile range 19 years), with male: female ratio of 1.4: 1. The maximum number of patients (42.5%) were in ELTS intermediate-risk group, followed by ELTS high-risk (32%) and low-risk (25.5%) groups. Treatment results for CHR & EMR at 3 months of 67% (n = 71) patients was available at time of analysis. Overall, 98% patients achieved CHR and 75% patients achieved EMR at 3 months. EMR was achieved in 85.7%, 75% and 68% patients in ELTS low-risk, intermediate-risk and high-risk groups respectively, the difference being statistically significant (p value 0.049). The baseline patient characteristics & early treatment results in the different ELTS risk groups are given in Table 1 & Figure 1.
Conclusions: In our single-center experience, majority (75%) of CML-CP patients were in ELTS intermediate-risk & high-risk groups. Although the complete hematological response rates were similar across the ELTS risk groups, achievement of early molecular response at 3 months of TKI treatment was significantly affected by baseline ELTS risk stratification.

Table 1: Patient characteristics, ELTS risk scores & treatment results in the study

Parameters	Low-risk n = 27 (25.5%)	Intermediate-risk n = 45 (42.5%)	High-risk n = 34 (32%)	p value
Median age (years) (Inter-quartile Range)	33 (19)	38 (19)	38 (20)	0.160
Male gender [n (%)]	17 (63%)	25 (55%)	20 (59%)	0.780
Median Hemoglobin (g/dl) (Inter-quartile Range)	9.90 (2.7)	10.2 (2.4)	8.35 (1.55)	0.164
Median Total leukocyte count (x 10^9/L) (Inter-quartile Range)	157 (214)	197 (218)	170 (203)	0.243
Median Platelet count (x 10^9/L) (Inter-quartile Range)	368 (226)	279 (265)	197 (255)	0.177
CHR at 3 months [n (%)]	14/14 (100 %)	27/28 (96.4 %)	28/29 (96.5%)	0.772
EMR at 3 months [n (%)]	12/14 (85.7 %)	21/28 (75 %)	20/29 (68 %)	0.049

Figure 1: Early treatment response at 3 months as per ELTS risk stratification in the study



A Case Of Polycythemia Vera With Normal Hemoglobin

Harika Padamata, Uday Yanamandra, Harshit Khurana, Gurpeet Kaur, Bhushan Asthana

Introduction: Polycythemia vera is a chronic myeloproliferative disorder characterized by increased red cell mass. It should be suspected in patients with elevated hemoglobin or hematocrit, splenomegaly, or portal venous thrombosis.
Aims & Objectives: To highlight the varied presentation of polycythemia vera.
Materials & Methods: A 56-year old male, alcohol consumer for last 10 years with no documented comorbidities presented with left upper quadrant, dull dragging type abdominal pain of 3 months duration

associated with unquantified weight loss, and intermittent fever of 2 months duration. Clinically he had massive splenomegaly with hepatomegaly suspected to be secondary to alcoholic liver disease.Ultrasonography confirmed hepatosplenomegaly with raised echotexture of the liver with no ascites. Contrast enhanced tomography abdomen revealed thrombosis of the left main portal vein, splenic vein and focal segmental thrombosis of main portal vein with enlarged spleen and collaterals in splenic hilum. On hematological evaluation, he had neutrophilic leukocytosis, and raised lactate dehydrogenase. However, his hemoglobin was 14.6 g/dL, hematocrit 48.5 and biochemical parameters were normal. In view of the above clinical scenario, he underwent a bone marrow biopsy that revealed hyper cellular marrow with panmyelosis with trilineage proliferation of hematopoietic elements and absence of marrow fibrosis, favoring polycythemia. His serum erythropoietin levels were low, and JAK 2 V617F mutation was detected. In view of Mentzer index 10.30, hemoglobin electrophoresis was done which showed low HbA2 (1.8%) hence mutation analysis was done which showed heterozygous deletion indicating Alpha thalassemia trait. He was managed with anticoagulation for thrombosis, ruxolitinib.

Result: We present an interesting case managed at our center who presented with hepatosplenomegaly, portal vein thrombosis and normal hemoglobin value with a final diagnosis of polycythemia vera with Alpha thalassemia silent.

Conclusions: This case highlights the occurrence of polycythemia vera with normal hemoglobin value due to concomitant alpha thalassemia heterozygous silent mutation.

Clinical Outcomes Of Allogeneic Hematopoietic Cell Transplantation In Myelofibrosis: A Single Centre Experience

Nutan Joshi, Uday Kulkarni, Sushil Selvarajan, Sharon Lionel, Anu Korula, Anup J Devasia, Fouzia NA, Kavitha M Lakshmi, Madhavi Maddali, Eunice Sindhuvi, Poonkuzhali Balasubramanian, Aby Abraham, Vikram Mathews, Alok Srivastava, Biju George

Introduction: There is paucity of data on allogeneic transplantation (allo-HCT) for myelofibrosis, especially from developing countries.

Aims & Objectives: To describe the clinical outcomes of transplantation in patients with myelofibrosis.

Materials & Methods: We performed a retrospective analysis using hospital records of all patients with myelofibrosis who underwent their first allogeneic transplantation at our center from January 1998 to June 2022.

Result: 27 patients underwent allogeneic transplantation for myelofibrosis. Median age was 47 (19–65) years, 20 (74%) were males. Median DIPSS Score was 3 (2–4). Of the 17 patients where data on all 3 driver mutations was available, JAK2 was most common mutation (8 patients). The karyotype was unfavorable in 4 (of 22 patients tested) while 3 (of 5 patients tested) had high risk molecular mutations. Table 1 describes the characteristics of the patients. All patients had peripheral blood grafts with donor source being matched sibling in 18 (66.7%) patients, matched unrelated in 6 (22.2%) and haplo-identical in 3 (11.1%). Median CD34 cell dose was 9.28 (3.42–22) $\times$ 106/kg. Most patients received reduced intensity conditioning with Fludarabine-Melphalan $\pm$ TBI (24 patients). Most patients received calcineurin inhibitor + methotrexate (23 patients) as GVHD prophylaxis.

Two patients died before day 14 of sepsis while 3 (11.1%) had primary graft failure and subsequently died of sepsis (1 died following a second transplant). Two patients had sinusoidal obstruction syndrome while one had thrombotic microangiopathy. Of the 22 patients who had neutrophil engraftment (median day of engraftment being day + 14 (8–25), acute GVHD was noted in 15 (68.1%) patients with 10 having grade 3–4 GVHD. Chronic GVHD was noted in 9 patients (of 14 evaluable patients) with 3 being extensive chronic GVHD as per revised Seattle criteria. The median follow up of surviving patients was 18.5 months. At last follow up, 15 (55.5%) patients have died (one patient progressed to AML after 8 years and died subsequently following a second transplant) and 12 are alive (44.4%) and in remission at median of 18.5 (2.6–212.2) months.

Conclusions: Allo-HCT for myelofibrosis is associated with reasonable cure rates however strategies to reduce graft failure and GVHD are required.

TABLE 1 : PATIENT CHARACTERISTICS , PRETARNSPLANT FACTORS AND OUTCOME (n=27)

Characteristic	Number (%) or median (range)
Age	47 years (19-65)
DIPSS Score	3 (2-4)
Male sex	20 (74%)
DRIVER MUTATIONS (Data available for 22 patients)	
1. JAK 2	8 (36.4%)
2. CALR	4 (18.2%)
3. Triple negative	5 (22.7%)
4. JAK Neg , CALR and MPL not tested	5 (22.7%)
PRIOR THERAPY WITH RUXOLITINIB	11 (40.7%)
PRIOR THERAPY WITH STEROIDS + THALIDOMIDE	18 (66.6%)
PRIOR THERPY WITH HYDROXYUREA	06 (22.2%)
DONOR SOURCE	
1. Matched Sibling donor	18 (66.6%)
2. Matched Unrelated donor	6 (22.2%)
3. Haplo-identical donor	3 (11.1%)
CD34 CELL DOSE	9.28 (3.42 - 22) x 10^6/kg
CONDITIONING REGIMEN	
1. Fludarabine-Melphalan	22 (81.5%)
2. Flu-Mel-TBI	2 (7.4%)
3. Busulfan-Cyclophosphamide	2 (7.4%)
4. Fludarabine-Cyclophosphamide	1 (3.7%)
GVHD PRPHYLAXIS	
1. Cyclosporine + Methotrexate	21 (77.8%)
2. Tacrolimus + Methotrexate	2 (7.4%)
3. Post-transplant Cyclophosphamide	4 (14.8%)
NEUTROPHIL ENGRAFTMENT	

Yes	22 (81.5 %)
Time to engraftment	Day +14 (8-25)
PLATELET ENGRAFTMENT	
Yes	17 (63%)
Time to engraftment	Day +17 (10-160)
GRAFT FAILURE	3 (All three primary)
COMPLETE DAY 28 CHIMERISM	19 (86.3%)
ICU CARE	14 (51.9%)
Acute GVHD	15 (68.1%)
Chronic GVHD	9 (64.2%)
REGIMEN RELATED TOXICITY	
Veno-occlusive Disease	2
Thrombotic Microangiopathy	1
STATUS AT LAST FOLLOW UP	
Alive	12 (44.4%) (All in remission)
Dead	15 (55.6%)
OUTCOME (ALIVE PATIENTS) AS PER DONOR SOURCE	
MSD (of a total of 18)	9 (50%)
MUD (of a total of 6)	3 (50%)
CAUSE OF DEATH	
GVHD	6 (40%)
Graft failure	3 (20%)
Sepsis	4 (26.6%)
VOD	1 (6.65%)
Relapse	1 (6.65%)
TRM at 1 year	12 (44.4%)

Real World Data On Use Of Ruxolitinib And Challenges Associated With It Inpatients Of Primary Myelofibrosis

Akash Khandelwal, Tulika seth, Manoranjan Mahapatra, Mukul Aggarwal, Rishi Dhawan, Pradeep kumar, Seema Tyagi, Jasmita Dass, Ganesh Kumar V

Introduction: Ruxolitinib has been used as a first line agent for primary myelofibrosis ever since results of comfort I and comfort II trials, before thisthe only drug treatment option was cytoreductive therapy like hydroxyurea or agents like thalidomide and prednisolone. Allogenic bonemarrow transplant remains the only curative option for these patients.

Aims & Objectives: To assess role of ruxolitinib vs other therapies in improving quality of life of patients of PMF.

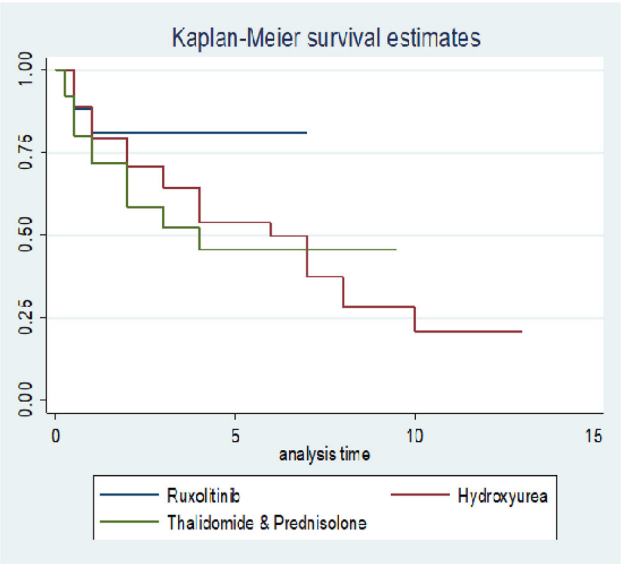
To assess side effects and challenges associated will ruxolitinib use.

Materials & Methods: This was an observational cross sectional ambispective study conducted in department of haematology between september 2020 to june 2022. 96 patients (39 newly diagnosed and 57 follow up patients) were enrolled in it. Treatment was not randomized but as ruxolitinib is a costly drug and not everyone can afford it patient were also offered other therapies like hydroxyurea and thalidomide regularly,hence automatically treatment groups were created. Disease burden via MPN 10 questionnaire was assessed, also spleen response was assessed at 24 weeks, presence of any side effects.

Result: Total 96 patients were enrolled 17 patients had been offered ruxolitinib 1st line, 22 patients were given ruxolitinib as second line i.e., post failure of other therapies (hydroxyurea or thalix prednisolone), ruxolitinib showed a better progression free survial as compared to other therapies (fig. 1).

Spleen response of $> 50\%$ at 24 weeks assesed via palpation was highest 67% with ruxolitinib as compared to 51% with hydroxyurea and 17%with thalidomide and pred respectively. Ruxolitinib group showed high Infection rates with 10(25%) patients had tuberculosis, severe grade infections in 7(18.5%) patients,transfusion dependent anaemia, (seen in 31% patients) thrombocytopenia (seen in 46% patients).

Conclusions: Ruxolitinib improves spleen related symptoms and improves disease burden but it's not curative, also it has nominal effect on disease,post progression. It can also be associated with many side effects. One must be vigilant post starting ruxolitinib for its side effects and complications. Other drugs like hydroxyurea or thalidomide and prednisolone can be offered in all non-affording group of patients and they can be effective in some patients, there effect can wary off and patients symptoms can reoccur, such patients can be easily challenged with ruxolitinib with an overall better response.



Mutational Landscape Of Myeloid Malignancies Unlocked By Next-Generation Sequencing: An Experience On 366 Patients From A Tertiary National Reference Laboratory

Ankita Jaiswal Govil, Aditi Agarwal, Sanjeev Kumar Sharma, Tina Bhardwaj, Rahul Katara, Deepak Kumar Sharma, Vipin Kumar, Rohit Yadav, Shivani Sharma, Sambit K. Mohanty

Introduction: Next-generation sequencing (NGS) has paved its path and broaden its utility in hemato-oncology practice. Myeloid malignancies are clonal mutations in hematopoietic stem cells and are subdivided into acute myeloid leukemia (AML), Myeloproliferative neoplasm (MPN), Myeloproliferative neoplasm/Myelodysplastic syndrome (MPN/MDS) and Myelodysplastic syndrome (MDS). NGS has the edge over single gene testing as it can aid in (a) massive parallel sequencing; (b) identifying deleterious genomic aberrations; (c) assessing baseline disease progression/recurrence and guidance to therapy; (d) conclude druggable tumor specific somatic mutations.

Aims & Objectives: This study aims at the implementation of a comprehensive NGS panel in myeloid malignancies. To evaluate the genomic profile of myeloid malignancies by NGS assay and ascertain its utility.

Materials & Methods: We evaluated a total of 366 consecutive patients over a two years period. Our panel enables to detect mutations across 40 key DNA mutation genes and RNA fusions across 29 driver genes relevant to major hematological disorders. The test utilizes Oncomine Myeloid Research Assay, which is based on the

proven Ion AmpliSeq technology. This assay allows concurrent analysis of DNA and RNA to simultaneously detect multiple types of variants, including hotspots, single-nucleotide variants (SNVs), indels, and gene fusions.

Result: 1. 210 of 366 (57.38%) tumors harbored pathogenic mutations.

2. We observed a total of 40 mutated genes that were clinically relevant.

3. Most frequently mutated genes included TET2 > FLT3 > ASXL1 > NPM1 > JAK2 > DNMT3A > NRAS > TP53 > IDH2 > IDH1.

4. Of these 40 gene mutations and fusions- PML::RARA (1.64%), FLT3 (8.74%), IDH2 (4.64%) and IDH1 (3.83%) were identified to have FDA approved targeted therapy.

Conclusions: Genomic assay aids clinicians in personalizing drug regimens by understanding tumorigenic pathways and timely management of the disease. This assay has helped to identify clinically actionable mutations allowing targeted therapy in complex myeloid malignancies. Comprehensive genomic profiling improves the evaluation of low to moderate penetration genes that are frequently overlooked in clinical genetic settings, resulting in a direct influence on providing individuals with appropriate genetic counselling and clinical care. It can be used to complement morphologic, immunophenotyping and cytogenetic work up.

Study Of CML Patients Receiving Branded Generic Imatinib Free From Government Supply With Reference To Compliance And Efficiency

Ritik Kumar Das Mohapatra, Sudha Sethy, Jayant Kumar Panda, R.K Jena

Introduction: Imatinib including a branded generic form has changed the landscape of CML. Its availability in govt. setup free of cost is a significant positive step. However regular medical evaluation with molecular monitoring and early switching over to second generation of TKI is a big challenge in resource constraint setup.

Aims & Objectives: Compliance of patients taking Imatinib.

Frequency of molecular monitoring and achievement of MMR at the end of 12 months as per ELN2022 criteria.

Progression free survival.

Materials & Methods: It is a prospective study in clinical Hematology Department of SCB Medical College Cuttack, Odisha from July 2021 to June 2022 involving 104 confirmed cases of CML receiving branded generic Imatinib free from Govt. Supply.

Compliance and molecular monitoring was studied by taking proper history and efficiency to treatment was judged on the basis of clinical examination, Hematological response and molecular responce at the end of one year by RTQPCR as per ELN 2022. PFS was determined in all cases at the end of 1 year.

INCLUSION CRITERIA

Confirmed cases of CML in chronic phase receiving branded generic Imatinib free from Govt. supply.

EXCLUSION CRITERIA

CML in Blast Crisis/Accelerated phase.

Result: Out of 104 CML patients, 73 patients had good compliance to Imatinib (70.19%). Molecular monitoring adhering to ELN 2022 is depicted in the below table. Molecular monitoring at 3 months was 7.6% and 6 months was 5.7%. Imatinib failure in 1 year was 39.42%

Time Points (Month)	Frequency of monitoring (No./%)	No. of patients achieved MMR	Percentage of patient achieved MMR @1 yr	Percentage of patient with treatment Failure
3	08(7.6%)	–		
6	06 (5.7%)	–		
12	46(44.23%)	30	65%	41(39.42%)

Conclusions: • Adherence to Imatinib treatment is 70.19% even if the drug is available free.

• Molecular monitoring is significantly low i.e. 7.6% at 3 months, 5.7% at 6 months and 44.23% at 1 year.

• Imatinib failure is detected in 39.42% patients.

Significance Of Additional Chromosomal Abnormalities In Patients Of CML: An Observational Study

Priyanka asawa, Sudip roy

Introduction: Additional chromosomal abnormalities are considered high risk features in CML. The emergence of additional chromosomal abnormalities in BCR-ABL positive CML is considered to be a feature of disease evolution.

Additional chromosomal abnormalities (ACAs) are thought to result from BCR-ABL1-induced genetic instability and may be causative factors of disease progression. The most frequent ACAs found in BC (+ 8, a second Ph-chromosome (+ Ph), an isochromosome of the long arm of chromosome 17, i.e., i[17q], and + 19) were termed major route abnormalities.

Aims & Objectives: To study significance of additional chromosomal abnormalities in patients of CML.

Materials & Methods: This prospective study was carried on 200 patients conducted at IHTM Kolkata for a period of 1 month (August 2022). Detailed evaluation of patients was done with cytogenetic and bone marrow aspiration reports.

Result: Total three patients were found with additional chromosomal abnormalities. One having additional chromosomal abnormality of trisomy 8 was found to be in accelerated phase de novo. Other having deletion 4q before therapy which was not found after 3 month of therapy. While third one had additional chromosomal abnormality of trisomy 8 was found to have TKI resistance.

Conclusions: Additional chromosomal abnormality is a rare finding in CML and is usually associated with worse prognosis.

Miscellaneous

Role Of Fibrinogen, D-Dimer And Interleukin-6 In Type 2 Diabetes Mellitus

Kanika Raturi, Vijay Kumar, Bindu Kulshrestha, Neera Sharma, Akansha Bhatia, Neha Singh

Introduction: Diabetes Mellitus(DM) is one of the most common metabolic disorder having persistent hyperglycemia with long term complication leading to significant morbidity and mortality. Apart from hyperglycemia, various prothrombotic and inflammatory biomarkers have a role in DM and its complications.

Aims & Objectives: To estimate levels of fibrinogen, d-dimer and interleukin-6 in patients of type 2 diabetes mellitus and to correlate its level with glycemic control.

Materials & Methods: This study is an observational cross sectional study which was done between january 2021 to may 2022 at ABVIMS &Dr RML Hospital,New Delhi.In this study 70 patients were taken along with age and sex matched control. After obtaining a valid consent from those patients who met the inclusion criteria, they

were subjected to a detailed history and clinical examination. These patients were investigated for Complete blood count, Fasting/post prandial blood sugar, Lipid profile, HbA1c, D-dimer, Fibrinogen and IL-6. Levels of above parameters were compared between cases and controls.

Result: This study was conducted on patients of age group 35 to 70 years with newly diagnosed type 2 DM, or with known case of type 2 DM without complications and these patients were included as cases. A separate group of healthy individuals of same age and sex was enrolled as controls. Fibrinogen level of cases was significantly higher (373.91 ± 77.69 mg/dL) as compared to control (279.11 ± 58.23 mg/dL). Similarly D-dimer level was also found to be significantly higher in cases [241.5 ng/mL (200–300 ng/mL)] as compared to control [200 ng/mL (180–222 ng/mL)]. Levels of IL 6 were also significantly higher [10.18 pg/mL(9.09–12.40 pg/mL)] in cases as compared to control [8.0 pg/mL (7–9 pg/mL)].

Conclusions: Diabetes Mellitus is a pro-coagulant and low grade inflammatory condition as evident from our study as the levels of coagulation and inflammatory markers such as fibrinogen, d-dimer and interleukin-6 are significantly increased in the patients of Diabetes Mellitus even without complications. This study highlights the significance of these inflammatory markers as significant prognostic biomarkers in Diabetes Mellitus.

Haemoglobin D Iran In West Bengal: A Report Of Three Cases

Sunistha Bhattacharjee, Moupali Ghosh, Jyoti Shaw, Maitreyee Bhattacharyya

Introduction: The haemoglobin (Hb) D Iran, a rare Hb variant, occurs mainly in north- west India, Pakistan and Iran. The heterozygous and homozygous both forms of the disease is clinically silent.

Aims & Objectives: The aim of this study was to confirm the HPLC impression of three individuals by beta globin gene sequencing.

Materials & Methods: Three individuals visited IHTM, Medical College Kolkata for thalassemia screening. 2 ml of blood samples in EDTA vial was collected with informed consent form. Samples were initially subjected to Complete Hemogram analysis (Sysmex KX- 21), HPLC analysis (Bio-Rad Variant II, Beta Thal Short Program). Further Sanger sequencing (ABI 3500 genetic Analyzer) was done to confirm the mutation in beta globin gene.

Result: First patient was an elderly primigravidae presented with persistently low Hb even after oral hematinics, no other clinically relevant features, second patient a 37 year old male came for prenatal Hb-HPLC screening. Third patient was a college student visited IHTM for routine thalassemia screening. HPLC analysis of these three patients revealed combined HbA2 + E peak as 40.50%, 41.20%, 43.5% respectively leading to a suspicion of a heterozygous state. Sequencing analysis shows the presence of Hb D Iran in all the three cases. This mutation is located at codon 22 of exon 1 region in beta globin gene. This G > C mutation is responsible for Glutamic acid to Glutamine amino acid change.

Conclusions: Thalassemia screening of population by HPLC leads to rare disorders which may be confirmed by DNA sequencing.

Name	Age	Sex	Hb	RDW	MCV	MCH	HbA0	HBF	HbA2 + E
Patient 1	37	MALE	13	13.6	81.7	25.9	49.5	0.1	41.20
Patient 2	27	MALE	13.4	13.5	79.7	26.1	54.4	0.1	43.5
Patient 3	38	FEMALE	10	15.4	82.4	25.9	54.7	0.3	40.50

Clinical History of Three Individuals

Studies On The State Of Iron Nutrition In Pregnant Women And Lactating Mothers Of Murshidabad District In West Bengal

Smritiratan Tripathy, Priyanka Das

Introduction: Iron is a fundamental micronutrient, present within haemoglobin of red blood cell, which play an important physiological role in oxygen transportation and energy formation. Our body cannot synthesize iron, so, it must be acquired. The only natural source of iron is food supplements. Although the human body have the mechanism to recycle and reutilize iron, but it loses some amount of iron daily. The most of the iron need in our body is fulfilled by the process of recycling the iron from senescent red blood cellswith the help of macrophages. Dietary foods can fulfil only 5 to 10% of iron requirements in our body. The demand of iron remains high at certain stages of life mostly in growing children, adolescent girls, pregnant women and lactating mothers. The depletion or inadequacy of iron in our body results anemia with lower haemoglobin level and decreased red blood cell count.

Aims & Objectives: The objectives of the study were to evaluate the state of iron in pregnant women and lactating mothers i.e., most vulnerable group of the society in the socioeconomically retarded population in certain selected areas of Murshidabad district of West Bengal.

Materials & Methods: In this study, 88 pregnant women and 56 lactating mothers were recruited from Murshidabad district of West Bengal. About 1 ml of venous blood was collected from them and carried out some biochemical parameters such as red blood cell count and haemoglobin concentration. The statistics were done using student's t-test.

Result: The results were depicted that 6.81% of pregnant women and 5.36% of lactating mothers having their haemoglobin level less than 7 gm% and 10.23% of pregnant women and 7.14% of lactating mothers having theirred cell count less than 3.5 million/mm3. These biochemical indices clearly indicate anemia.

Conclusions: This study indicates that a large number of pregnant women and lactating mothers of Murshidabad district of West Bengal are suffering from mild to severe degree of anemia with lower haemoglobin level and decreased red blood cell count.

Can Plasmodium Vivax Malaria Lead To Hypokalemic Quadriparesis?

Ginni Bharti, Tushar Sehgal, Prachi Mohapatra, Mamta Bhushan Singh

Introduction: Hypokalemic periodic paralysis is an uncommon condition. Acquired and genetic causes have been identified. Here, we describe a 25-year-old man who presented with high-grade fever and acute-onset quadriparesis. Temporally, the weakness followed treatment with intravenous dextrose. A fever workup revealed infection with Plasmodium vivax, and biochemical analysis also uncovered low serum potassium levels. This was the first episode of quadriparesis in the patient, and it remains speculative whether this hypokalemic

periodic paralysis was precipitated by intravenous glucose or a rare systemic manifestation of malaria.

Aims & Objectives: A 25-year-old male was brought with complaints of high-grade intermittent fever with chills for seven days. After a few days of the fever, he went to a local doctor, who gave him two bottles of intravenous dextrose. He then developed sudden onset of quadriparesis that evolved over 48 h to a stage where he could not move. His temperature was 102.5° F, pulse rate of 92/min and BP of 109/65 mm Hg. On neurological examination, the patient had a bilaterally symmetrical pure motor quadriparesis of MRC Grade 2–3/5, with proximal weakness being more than the distal.

Materials & Methods: CBC revealed a haemoglobin of 13.2 g/dl, a WBC of 4.71 × 10^9/L and a platelet count of 30 × 10^9/L. Blood film revealed the trophozoite and gametocyte forms of Plasmodium vivax malaria. Among the electrolytes, serum potassium was 2.7 mmol/L. I.V potassium chloride 40 mEq was given. The patient's quadriparesis had recovered completely. The patient received intravenous artesunate 120 mg, 12 hourly for 5 days.

Result: Musgrave first described hypokalemic periodic paralysis (HypoKPP) in 1727. Most cases are hereditary or familial. Acquired cases are associated with hyperthyroidism, renal tubular acidosis, gastroenteritis, or endocrine causes. Motor weakness due to malaria has been rarely reported, like in a 26-year-old male with P. vivax who developed hypokalemic paraparesis. The pathophysiology behind developing hypokalemic HypoKPP in various infections is not well understood.

Conclusions: All cases of HypoKPP must be evaluated thoroughly to exclude secondary causes, and motor weakness secondary to complicated infectious diseases must also be kept in mind, especially by physicians in endemic areas, for an early diagnosis and timely treatment.

Annual Audit Of Bone Marrow Examination

Anita Tahlan, Sourabh Kumar,Anshu Palta,Sanjay D Cruz

Introduction: Bone marrow may be involved and sometimes affected in conditions that for a broader part arepredominantlyhaematological. Though non-haematological disorders may also affect the bone marrow. Haematological disorders both benign and malignant, including, but not limited to, acute leukemia, myeloproliferative neoplasm (MPN), lymphoid neoplasm, nutritional deficiencies,infections,etc. On the other hand, non-haematological indications or disorders like staging for a haematological or systemic disease like lymphomas or infectious diseases infiltrating the bone marrow such as tuberculosis, parasitic infections and metastatic deposits.1 Although, diseases of bone marrow present with various clinical symptoms and also involve the blood but peripheral blood picture alone does not reflect the nature of disease process. Depending upon diagnosis suspected from the clinical features and peripheral blood examination, the procedure of bone marrow examination comprising of a bone marrow aspirate and/or trephine biopsy is indicated.2 In this audit we have tried to analyse the spectrum of bone marrow findings in our setup which is a tertiary care hospital and study the utility of the procedure of bone marrow examination for diagnosis, treatment response assessment and subsequent management in haematological and non-haematological disorders.

Aims & Objectives: To analyse the morphological spectrum for bone marrow examination. and correlate with clinical diagnosis.

Materials & Methods: All patients who underwent a bone marrow examination including an aspiration and bilateral trephine biopsy at Department of Pathology, Government medical college and hospital, Sector-32,Chandigarh from January 2018 to December 2018 were included in the study. The cases refereed from outside for review were excluded. Patient age, sex, clinical history, indication for the procedure and provisional clinical diagnosis were recorded. After routine haematological investigations, bone marrow specimens were obtained from the posterior iliac crest in all patients according to standard technique.Complete blood counts were done. Peripheral and bone marrow smears were prepared and stained by wright-Giemsa stain while trephine biopsies were decalcified and paraffin embedded blocks were stained with usual haematoxylin and eosin (H&E) stain and examined. Appropriate marrow immunohistochemical, and reticulin stains were used where necessary.

Result: We analysed 322 bone marrow aspirate and trephines in all age groups and both genders in a period of one year. The dataset obtained was categorized into Paediatric (0-18yrs), Adult(19-60yrs) & Geriatric (> 60yrs). Each of the category was further classified into Haematological and non-haematological. Haematological was further divided into benign and malignant.. There were 180 males (55.9%) and 142 females (44.1%) the M: F ratio was1.2:1.The age of the patients ranged from 7 months to 90 years (Table 1). Majority of our patients were in Group II (70.5%) in the age group II i.e. 19–60 years.

Conclusions: Bone marrow examination is an invasive procedure yet it gives significant information and should be practiced judiciously. This examination still is a pivotal for diagnosis, response assessment and subsequent management in haematological and non-haematological disorders alike especially in a developing country like ours.

Survey Of Bone Marrow Study Done At A Tertiary Care Centre In Coastal Karnataka

Monika J Gamit, Gloria Venessa Quoadros, Indira Puthran, Rajesh Krishna

Introduction: Bone marrow examination is an essential investigation for diagnosis & management of many disorders of blood & bone marrow.

Aims & Objectives: To study the indications of Bone marrow study along with spectrum of diagnosis in tertiary care hospital.

Materials & Methods: This study was carried out in Yenepoya Medical College Hospital, Mangalore, Karnataka for a period of 2 years from July-2020 to July-2022. Records regarding clinical indication, age & gender, diagnosis of BM study & requesting department was retrieved from hospital's electronic data system.

Result: We studied 406 consecutive Bone marrows performed during this period. Male: Female ratio in our study was 1.5:1.Out of 406,66 BM were between the age group of 1–20 years, 120/406 BM were between age group of 21–40 years, 135/406 were between the age group of 41–60 years & 85/406 BM were > 60 years. Most common indication was pancytopenia accounting for 72 cases followed by suspicion of Multiple myeloma (56),suspicion of acute leukaemia(37), suspicion of chronic leukaemia(12), evaluation of anaemia(30),suspicion for HLH(20),suspicion of ITP(11), suspicion of MPN(19),suspicion of MDS(14), (22) suspicion of marrow infiltration, (22)BM were done to know the phase of disease, suspicion of metastasis(6) & suspicion of Amyloidosis (3).BM study yielded diagnosis of malignancy in 102 cases including AML(27),ALL(22),CML(13),CLL(8),MM(26) and metastasis(6). 131 cases diagnosed as benign including increased peripheral destruction(75), HLH(34), IDA(8),Megaloblastic anaemia(14).Dry tap noted in 28 cases.141 Bone marrows were requested by department of Oncology followed by Medicine(131),Paediatrics(37),Surgery & Orthopaedic(15). Acute leukaemia was diagnosed in 59 cases with most common age group affected was 0–20 years &requesting departments were haemato-oncology(35) followed by paediatrics(17) & Medicine(7).Chronic leukaemia was diagnosed in 22 cases with most common age group affected was > 60 years, requesting departments were Haemato-oncology(16) & Medicine(6).Multiple Myeloma was diagnosed in 23 with most common age group affected

41–60 years& requesting departments were haemato-oncology(8),orthopaedic(6) & medicine(7).

Conclusions: Our survey showed that vast majority of bone marrow study performed were diagnostic. Majority of requests were from haemato-oncology followed by medicine.The most common diagnoses remain Benign followed by malignant disorders Bone marrow aspiration is valuable diagnostic tool in diagnoses of various haematological findings.

A Landscape Of Bone Marrow Metastasis: Unraveled By Various Procedure

Sneha Chauhan, Veenu Jain, Priyanka Rai, Kamlesh Kumar, Mamta

Introduction: Bone marrow, the site of origin of numerous primary haematologicalmalignancies, is commonly involved by metastatic tumours. The presence of metastases in the bone marrow usually means worse prognosis. It is considered imperative to rule out marrow involvement in any malignancy where curative treatment is being considered.

In adults the tumours seen are carcinomas of the prostate, breast and lungs, although any tumour of children like neuroblastoma, rhabdomyosarcoma, Ewing's sarcoma and retinoblastoma account for the majority of metastases.

Aims & Objectives: 1.To know the relative incidence of bone marrow involvement by various malignancies.

2.The merits and demerits of various procedures.

Materials & Methods: This study was carried out on 109 patients adults and children attending outdoor and admitted in indoor wards of surgical oncology with clinical suspicion or histopathological diagnosis of various malignancies or suspected of having metastatic neoplasms.

Result: 109 patients in which bone marrow procedures were done, marrow metastasis was present in 42 cases.

42 cases in which marrow involvement was present, 8 cases (19.0%) were from non- haematological malignancies, 24 cases (57.2%) were from non- Hodgkin's lymphoma and 5 (11.9%) each from Hodgkin's lymphoma and multiple myeloma.92.8% aspirates were diagnostic for marrow metastases.

90.4% clot sections could diagnose the presence of classifiable metastases in the bone marrow.

42 cases in which marrow metastasis was found to be positive, trephine biopsy and imprints could be performed only in 30 patients (71.4%)

Evidence of marrow metastasis was found in all above cases of trephine biopsies and imprints performed (100%).

Out of these, 3 cases (10%) could be diagnosed by trephine biopsy and imprints only.

Conclusions: The incidence of bone marrow metastasis by various malignancies was 38.5%.

The relative incidence of bone marrow involvement by various malignancies was.

Non-Haematological malignancies 19.0%

Non-Hodgkin's lymphoma 57.2%

Hodgkin's lymphoma 11.9%

Multiple myeloma 11.9%

34.8% (8/23) cases of non-haematological malignancies were found to be positive for marrow metastasis.

In our study most common primary sites for bone marrow metastasis in adults were prostate and breast while in children, neuroblastoma and retinoblastoma were most common primary sites.

Etiological Spectrum Of Pancytopenia, On Bone Marrow Examination: An Observational Study

Indrani Mondal, Maitreyee Bhattacharyya, Debdas Bose

Introduction: Pancytopenia describes simultaneous presence of anemia (Hb $<$ 10 g/dl) leucopenia($<$ 4000/cmm) and thrombocytopenia($<$ 150,000/cmm).It's not a disease itself but is a condition resulting from a number of disease processes. Different etiological factors are responsible for pancytopenia- Aplastic anemia, megaloblastic anemia, leukemia, etc. This study was carried out to identify the causes of pancytopenia, to find out the frequency of different causes, to determine the etiology of pancytopenia and to compare our findings with those of other similar studies.

Aims & Objectives: This study was carried out to identify the causes of pancytopenia, to find out the frequency of different causes, to determine the etiology of pancytopenia and to compare our findings with those of other similar studies. To find out the reversible causes of pancytopenia so that we can treat early.

Materials & Methods: This was a prospective observational study conducted at IHTM, Kolkata for a 9-month period (Dec 2021 to August 2022). All the patients who came for Bone marrow examination are evaluated with detailed clinical history, peripheral blood smear and other ancillary investigation done prior to BM Examination. A total number of 100 cases that fulfilled the diagnostic criteria of pancytopenia were selected.

Result: A female preponderance was observed, the male to female ratio being 1: 1.04 Mean age was 44 years (range, 2-75yrs.). The commonest cause was hematological malignancy in 50cases (50%)-Acute leukaemia (23%), Chronic lymphoproliferative disorder(11%),MDS(10%),Plasma cell dyscrasia(4%),PMF(2%). Followed by Hypoplastic bone marrow seen in 25%, Megaloblastic anaemia 19% was seen in 6 cases (13%) and normal reactive bone marrow was seen in 2%. Acute leukemia was the commonest hematological malignancy.

Conclusions: Pancytopenia is a common occurrence. Acute leukemia and Hypoplastic marrow were the common causes of pancytopenia. Megaloblastic anemia is the reversal cause of pancytopenia.

Compound Heterozygosity For Sickle Cell Hemoglobin And Hemoglobin D-Punjab (D-Los Angeles): A Rare Entity

Neha Singh, Akanksha Bhatia, Garima Baweja, Vijay Kumar

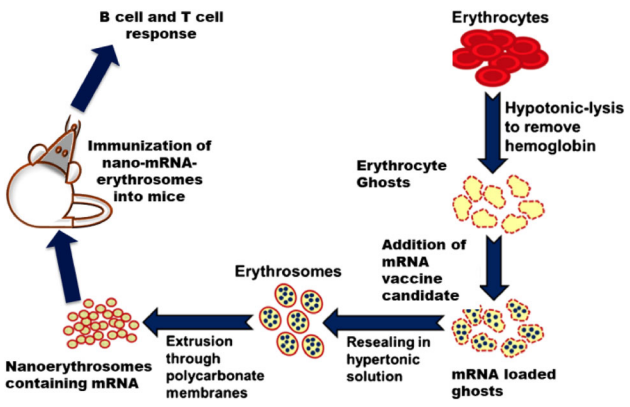
Introduction: Case report: Haemoglobinopathies constitute a major burden of the inherited hematological disorders worldwide. Sickle cell disease constitutes one such disorder caused by a structural variant of hemoglobin that damages and deforms red blood cells and affects multiple organ systems. It is categorized as SS, Sickle cell/HbC, Sickle cell/Hemoglobin D-Punjab (Hemoglobin D-Los Angeles), SO-Arab, S-β-thalassemia, S-hereditary persistence of fetal hemoglobin and SE. Among these, Hemoglobin S/Hemoglobin D-Punjab (Hemoglobin D-Los Angeles) is a rare compound heterozygous hemoglobinopathy characterised by interaction of hemoglobin S with Hemoglobin D-Punjab (Hemoglobin D-Los Angeles) and the coexistence of two globin gene variants: 6(GAGGTG) and 121(GAACAA). We present here one such rare case of a young boy who presented with intermittent fever and multiple joint pains and diagnosed to have Sickle cell/Hemoglobin D-Punjab disease on high performance liquid chromatography (HPLC) and confirmed by parental studies.

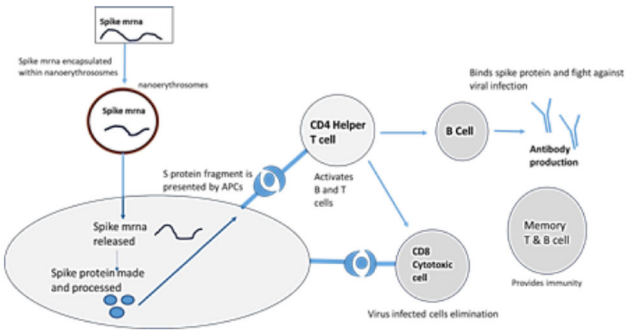
Key words: Hemoglobin, Sickle cell, HPLC.

Red Blood Cell-Derived Nanoerythrosomes Mediated Efficient Delivery Of Mrna Vaccine Candidate Against COVID-19

Shreeja Biswas, Swati Garg, Prerna Joshi, Oinam Ningthemmani Singh, Milan Surjeet, Pramod Garg, Anand Ranganathan, Shailja Singh

Introduction: The COVID-19 pandemic has been a major public health concern throughout the world. Various ventures of vaccine candidates are being studied rigorously in this regard and one such candidate is the receptor binding domain (RBD) of spike protein which interacts with angiotensin converting enzyme 2 (ACE2) on the host cell's membrane. Exploiting this interaction, many scientists across the world attempted to develop mRNA vaccine against SARS-CoV-2. A major lacuna associated with mRNA vaccines is their delivery through a suitable carrier, especially given the stability issues associated with mRNA vaccines.
Aims & Objectives: The aim of our research is to develop an efficient mode of delivery of the self-amplifying mRNA (saRNA) against COVID 19. We have developed small vesicles from erythrocyte ghosts, known as nanoerythrosomes, which are in the nanometre range and focussed on development of nanoerythrosomes for delivery of mRNA-based vaccines.
Materials & Methods: Nanoerythrosomes were prepared from erythrocytes using osmotic and ultrasonic frequency stress and loaded with saRNA vaccine candidate. Thereafter, the nanoerythrosomes were characterized using Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) to confirm their homogeneity, integrity and size. The mRNA loaded nanoerythrosomes were used to deliver the mRNA in Vero E6 cells to evaluate its uptake.
Result: The characterization of nanoerythrosomes using DLS and TEM revealed their size in the range of 100–200 nm. The delivery mediated by nanoerythrosomes was comparable to the Lipofectamine mediated uptake of saRNA indicating the excellent delivery efficacy of nanoerythrosomes. The added advantage of nanoerythrosomes mediated delivery is that they are rapidly taken up from blood by macrophages of the reticuloendothelial system (RES) that is present in liver, lung, and spleen. Thus the combination of saRNA and nanoerythrosomes can accelerate the uptake and antigen presentation in reticuloendothelial system and will provide an outstanding platform for the development of SARS-CoV2 vaccine.
Conclusions: We developed a new approach to deliver mRNA vaccine candidates using nanoerythrosomes and successfully demonstrate its efficacy in vitro. This strategy can be further extended for the delivery of other vaccine candidates.





Spectrum Of Histiocytic Disorders Of Bone Marrow: Diagnostic Enigma: A Case Series With Review Of Literature

Pushpit Gupta, Sarika Singh, Garima Rakheja, Bembem Khuraijam, Lity Dhar, Rabish Kumar, Suresh Kumar, Monica Juneja, Sunita Aggarwal

Introduction: Histiocytes are immune cells found in tissues throughout the body with diverse functions which include housekeeping via phagocytosis, activating immune system & promoting peripheral tolerance. Histiocytic disorders can be primary or secondary. WHO classified histiocytoses into 5 major groups: (1) Langerhans related (L group), (2) cutaneous and mucocutaneous histiocytoses (C group), (3) malignant histiocytoses (M group), (4) Rosai–Dorfman disease (RDD) (R group) and (5) haemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome (H group). The H group includes Hemophagocytic lymphohistiocytosis (HLH), a life-threatening syndrome of excessive immune activation mostly affecting infants from birth to 18 months of age but affects all ages. HLH can be primary (familial) or secondary (acquired).
Aims & Objectives: To highlight the role of diagnostic challenges and ascertaining the etiology for better management and understanding a complex disorder of this magnitude.
Materials & Methods: This was a retrospective study of 19 months duration (February 2021–August 2022). 350 marrow samples were retrieved from archive of Department of Pathology, MAMC. Peripheral blood & bone marrow examination (BME) of all cases was done. Blood samples were run on automated haematology analyzer (XN1000i) & complete blood count done. Bone marrow aspirate and biopsy were looked for histiocytes for evidence of hemophagocytosis. Biochemical tests like lipid profile, serum fibrinogen, ferritin & CD25 [removed]through flow cytometry) along with detailed clinico-haematological correlation.
Result: 34/350 (9.7%) cases were found to be histiocytic disorders. Out of these, 8/34 cases (23.5%) were reported as HLH, 3/34 (8.8%) as histiocytosis secondary to malignancy, 3/34 (8.8%) as reactive histiocytosis, 18/34 (53%) as histiocytosis secondary to infections & 2/34 (5.9%) cases as histiocytosis with unidentified cause. Age of patients ranged from 1 to 76 years with M:F ratio of 2.1:1, presented with fever & organomegaly of variable size. Hb levels varied from 4.5 to 15.3 gm/dl with Anemia (Hb < 9 gm%) in 22 cases & absolute neutrophil counts $< 1000/mm3$ in 7 cases. BME revealed variable cytopenia, hypo-normocellular marrow with mild to moderate lymphohistiocytosis and features of hemophagocytosis in these cases. Further workup for causal association was done & will be discussed.
Conclusions: Study of spectrum of histiocytic disorders is essential because of its variable presentation & multimodal causation, thus posing diagnostic challenges. Complete workup of clinico -pathological, biochemical and radiological parameters of patient is necessary for appropriate management.

Seroconversion After COVID 19 Vaccination And Disease In Adults With Hematological Diseases

Deepika Gupta, Nitin Gupta

Introduction: Patients with hematological diseases have impaired humoral immunity secondary to disease itself and due to its treatment. Treatment can be either steroids, chemotherapy and chemoimmunotherapy. The immune response to covid 19 vaccine or disease may be significant impaired in haematological diseases.
Aims & Objectives: None of the approved vaccine available in India (Covaxin, covishield and sputnik) has been approved for hemato oncology and transplant patients but most of our patients had received either of these vaccines as per national recommendations. Hereby we did retrospective study to look for antibody response in haematological diseases.
Materials & Methods: We retrospectively analysed the serological response to covid 19 disease and vaccination in 50 patients. Patients were divided into myeloid malignancies (Acute myeloid leukemia and MDS) n = 11, lymphoid malignacies n = 12 (CLL N = 8, Non Hodgkin Lymphoma n = 3, Pre B ALL n = 1), plasma cell dyscrasis (n = 8) (Multiple myeloma n = 6, AL amyloidosis n = 2), ITP N = 10 (patients on steroids, rituximab), MPNs n = 6 (CML N = 5, ET N = 1), AIHA N = 3. The complete history including the history of covid 19 diseases and covid vaccination was taken. Treatment history of the patient and antibody formation was analysed.
Result: SARS COV2 IgG antibody was tested in a total of 50 patients. Overall antibody response was present in 90.1% patients. The total igG antibody in myeloid malignancies was 482.45 (12–2000) u/ml, while MPN, lymphoid malignancies, plasma cell dyscrasis had median antibody 140.04 u /ml (0.2–250), 47.9.9u/ml (3.04–200), 1416.9u/ml (20–40,000) respectively. 2 patients with plasma cell dyscrasis and 2 patients with CML had no antibody formation. The benign disorders including itp on steroids and rituximab had median antibody of 262.72 u/ml (6.54—1358) and AIHA had 281.6 (45—400) u/ml. The results are shown in table 1.
Conclusions: This Study demonstrates low immunogenicity, mainly in patients with lymphoproliferative disorders, as well as with certain drugs, including mainly anti-CD20 antibodies, Bruton tyrosine kinase inhibitors. However, better humoral response rates are seen in plasma cell dyscrasis on treatment.

GROUPS	NO.OF PATIENTS (N=50)	COVID 19 disease	Covaxin	Covishied	Sputnik	No vaccine	Median IgG U/ML LEVELS
Myeloid	11	4	3	3	0	0	482.45 (12-2000
LYMPHOID	12	8	3	9	0		47.9 (3.04-200)
PCD	8	2	3	4	1		1416.9 (20-4000)
MPN	6	3	1	4	0	1	140.04 (0.2-250),
ITP	10	5	3	3		4	262.72 (6.54-1358)
AIHA	3	3	2			1	281.6 (45-400)

Evaluation Of Diagnostic Efficacy Of Novel Red Blood Cell Parameters As Potential Screening Test For Detecting Latent Iron Deficiency In Blood Donors

Abhishek Shukla, Namrata P. Awasthi, VK Sharma, Vandana Tiwari, Pradyumn Singh, Nuzhat Husain

Introduction: Novel RBC Parameters (NovRBC) are the new parameters available in advanced hematology blood counters which are considered research parameters at present. The purpose of this study was to assess the diagnostic efficacy of NovRBC parameters, specifically the percentage of hypochromic RBCs (%HPO), percentage of microcytic RBCs (%MIC), and the hemoglobin content of reticulocytes (MCHr), as predictors of latent iron deficiency (LID) in blood donors.
Aims & Objectives: The purpose of this study was to assess the diagnostic efficacy of NovRBC parameters, specifically the percentage of hypochromic RBCs (%HPO), percentage of microcytic RBCs (%MIC), and the hemoglobin content of reticulocytes (MCHr), as predictors of latent iron deficiency (LID) in blood donors.
Materials & Methods: This Cross sectional study for diagnostic test evaluation was conducted at RMLIMS, Lucknow. Total 260 blood donors were enrolled as per inclusion–exclusion criteria and informed consent was taken. All the donors had hemoglobin > 12.5 g/dl. EDTA and serum samples were collected. Complete blood count (CBC) with novel RBC parameters were performed on Abbott Alinity Hq analyzer within 2 h of blood collection. Serum Iron profile was done on Cobas 200 biochemistry analyser. Donors with serum ferritin < 30 ug/l and/or TSAT $< 15\%$ were considered to be having LID and the rest as normal.
Result: There were 205 males and 55 females. Proportion of LID/Normal was 34/171 (19.88%) and 22/33 (66.6%) in males and females respectively. Table 1 shows the details of results. As expected there was no significant difference in the mean values of Hb, HCT, MCV, MCH, MCHC and RDW in the two groups. However, we did not find any significant difference in the means of %MIC, %HPO and MCHr values as well.
Conclusions: To the best of our knowledge, this is the first study for evaluation of NovRBC parameters provided by Abbott Alinity Hq hematology analyser for detection of LID. We did not find any usefulness of %MIC, %HPO and MCHr for detecting LID in blood donors. Similar parameters have been studied on Sysmex series of analysers. %Hypo-He at a cut of 0.6% has been reported to be useful for detecting LID with sensitivity of 74.51% and specificity of 88.51%. As cellular changes in frank IDA are more pronounced than in LID, we suggest to evaluate the role of NovRBC parameters in frank Iron Deficiency anaemia on Alinity Hq before applying it for detecting LID.

Outcome Of Holistic Approach In Prevention Of Birth Of Child With Homozygous ?-Thalassemia And Sickle Cell Disease

Dolat Singh Shekhawat, Siyaram Didel, Abhishek Purohit, Charu Sharma, Pratibha Singh, Kuldee Singh

Introduction: Thalassemia and sickle cell disease (SCD) are the commonest monogenic disorders India contributes to about 15% of the world's sickle cell anemia neonates. Developed counties have controlled the birth of child with homozygous β-thalassemia and SCD. A holistic approach includes pregnant women and newborn screening, education, awareness, and genetic counselling can help to stop birth of with homozygous β-thalassemia and SCD children in India.
Aims & Objectives: To evaluate the birth of child with homozygous β-thalassemia and SCD using a holistic approach, in Rajasthan India.
Materials & Methods: Total of 1050 newborns was tested for hemoglobinopathies. Newborns blood was collected using standard DBS and samples were analysis using VARIANTnbs. 2 ml EDTA blood was taken from parents and first-degree relative of proband, complete blood count and HPLC for hemoglobinopathies was done. ARMS PCR was performed to analyze common mutation (IVS1-1 (G–T), VS1-5(G–C), CD41/42(-CTTT), CD8/9 (+ G), CD15(G–A) detection for thalassemia. Information, education and communication strategy were used to extensive awareness for hemoglobinopathies. Genetic counselling was done for thalassemia and sickle cell trait-positive cases.
Result: Out of 1050 newborns, 0.43% and 0.26% were shown hemoglobin D and E trait, respectively. For 0.17% newborn HPLC

result was intermediate (children are under follow-up). For hemoglobin D and E trait children, parental screening was significantly associated. Genetic counselling was done. Parents were educated about the carrier status and explained the risk of the disease. Carrier screening for the first-degree relative of proband and parents are under follow-up. All positive cases have been registered in the genetic patient registry at AIIMS Jodhpur.

Conclusions: Screening of newborn and first-degree relatives of proband and parents of the proband, genetic counselling, long follow-up, education, and awareness for β-thalassemia and SCD disease can effectively prevent the birth of homozygous β-thalassemia and SCD child.

Massive Splenomegaly And Hypersplenism As Rare Manifestation Of Adolescent Celiac Presentation

Kusum Lata, Kapil Bhalla

Introduction: Celiac disease is an immune-mediated disorder triggered by hypersensitivity to gluten in genetically predisposed individuals. A high index of suspicion is needed for diagnosis as patient can be asymptomatic or present with atypical symptoms or extra-intestinal manifestation. Splenomegaly in celiac disease has been described secondary to portal hypertension induced by antigenic stimulation of splenoportal axis and non-cirrhotic portal fibrosis.

Aims & Objectives:

Aim: To study association of celiac with hypersplenism.

Objective: To bring out rarity of association.

Materials & Methods: Here we report two newly diagnosed celiac cases with splenomegaly.

Case 1: 11 year old female child, presented with progressive pallor for last 3 months. On examination pallor, massive splenomegaly was noted. Her hematological parameters were: hemoglobin 3.2 mg/dl, TLC 1500/cmm, platelets 80,000 /cmm and retic count 4.46. SGOT, SGPT and ALP were 54, 22 and 113 U/L. Total bilirubin was 1.0 mg/dl (conjugated 0.3), portal vein diameter 8 mm, HPLC- normal study, bone marrow examination -normal and tissue transglutaminase IgA 186 U/ml. Based on clinical profile and laboratory findings, diagnosis of celiac disease was considered and gluten free diet was started.

Case 2: 14 year old female child, presented with pain abdomen, poor weight gain and weakness for last 7 months. On examination pallor, moderate splenomegaly was noted. Her hematological parameters were: hemoglobin 2.7 mg/dl, TLC 3200/cmm, platelets 20,000 /cmm and retic count 2.7. SGOT, SGPT and ALP were 125, 96 and 123 U/L. Total bilirubin was 1.2 mg/dl (conjugated 0.4).Portal vein diameter, thyroid function, stool examination, HPLC, bone marrow examination turn out normal and tissue transglutaminase IgA 233 U/ml. Diagnosis of celiac disease with pancytopenia and splenomegaly was made and gluten free diet and hematinic supplements were given.

Result: Follow-up after gluten free diet showed improvement in hematological parameters and regression of splenomegaly.

Conclusions: This case highlights the importance of considering celiac disease in patients with hematological abnormalities and splenomegaly with or without gastrointestinal symptoms. Celiac disease with hypersplenism is rare entity. Awareness of this entity may avoid misdiagnosis and guide appropriate management.

Post-Translational Modification Regulates Nmdar Channel Activity In Sickle Cell Disease Provides A Novel Therapeutic Strategy For Its Treatment And Malaria Protection

Geeta Kumari, Shailja Singh

Introduction: Sickle cell disease (SCD) is the most prevalent severe hemoglobinopathy in the world and an inherited, monogenic condition. A mutation in the globin gene produces Hemoglobin (Hb) S, which causes mechanistic and phenotypic alterations in sickle red blood cells.). SCD patients circulating RBCs contain an abnormally high number of functioning N-methyl D-aspartate receptors (NMDARs), which maintain the intracellular calcium (Ca2 +) level. Due to their high NMDAR content, sickle cells exhibit high Ca2 + permeability, causing oxidative stress and RBC dehydration. Oxidative stress causes post-translational modifications (PTMs) of proteins that contribute to disease progression. Similarly in NMDAR, PTMs such as phosphorylation and palmitoylation are essential for its channel activity. Elucidating PTMs regulation of NMDAR channel activity further facilitates the development of novel therapeutics for sickle cell disease.

Aims & Objectives: To study the PTMs in the regulation of NMDAR channel activity in the sickle and normal RBCs.

Screening of NMDAR antagonists library for potential channel blockers.

Effect of NMDAR antagonists on ROS generation and intracellular Ca2 + level.

Role of NMDAR antagonists in restoring normal phenotype and providing protection against malaria.

Materials & Methods: In this study, Acyl biotin exchange (ABE) and click chemistry were used to study the palmitoylation of NMDAR in the sickle and normal erythrocytes. We further screened the NMDAR antagonist library for potential channel disruptors. Furthermore, filtered-out antagonists were examined for their effects on NMDAR-dependent ROS generation and calcium levels in sickle cell RBCs. Further, these antagonists were tested for their antimalarial activity both in the sickle and normal erythrocytes.

Result: ABE and click chemistry analysis demonstrated palmitoylation-dependent channel activation of NMDAR membrane proteins and described their role in controlling intracellular Ca2 + levels inside sickle and normal RBCs. ABE study of sickle cell erythrocytes membrane revealed differential palmitoylation of membrane protein. Notably, NMDAR was observed to be actively palmitoylated. Screening of the NMDAR antagonist library revealed potent channel disruptors which reduce NMDAR-dependent ROS generation and Ca2 + level in sickle cell RBCs and aid in restoring the normal phenotype. These antagonists further inhibited the proliferation of malaria parasites in sickle and normal erythrocytes. Hence NMDAR antagonists not only correct the phenotype of sickle cell erythrocytes but also prevent growth of plasmodium.

Conclusions: Overall, this work highlighted the palmitoylation-dependent regulation of NMDAR channel activity in normal and sickle RBCs as well as the potential NMDAR antagonist which paves the way for a novel therapeutic strategy that protects against malaria infection while restoring the normal phenotype of sickle erythrocytes.

High Sensitivity Flow Cytometric Assay For Paroxysmal Nocturnal Haemoglobinuria Clone Detection: A Single Centre Experience Of 163 Cases

Tharageswari S, Nabhajit Mallik, Parveen Bose, Man Updesh Singh Sachdeva, Sreejesh Sreedharanunni, Praveen Sharma, Pulkit Rastogi, Narender Kumar, Shano Naseem, Prashant Sharma, Jasmina Ahluwalia, Reena Das, Pankaj Malhotra, Amita Trehan

Introduction: Paroxysmal Nocturnal Haemoglobinuria (PNH) is caused by somatic mutations in PIGA gene, leading to reduced synthesis of GPI anchored proteins. PNH clones may be detected in varying amounts in disorders like aplastic anemia and myelodysplastic syndromes (MDS). High-sensitivity flow cytometry assays can detect PNH clones as low as 0.01% with relative ease.
Aims & Objectives: To assess the detection of PNH clones by high sensitivity flow cytometry testing and their correlation with various hematological parameters.
Materials & Methods: Over a period of two and a half years (January 2020 to June 2022), a total of 985 cases were analysed by flow cytometry for detection of PNH clones. A single tube, 6 colour assay (CD45, CD15, CD64, FLAER, CD24, CD14) was used for WBCs, and 2 colour assay (CD235a/CD59) for RBCs. High sensitivity of the assay was ensured by acquiring at least 0.5 million (and up to 1.6 million) events per tube in all cases.
Result: PNH clones were detected in 163 of the 985 cases analysed. Age of patients ranged from 4 to 73 years (median 33 years), with 32 patients being less than 18 years of age. Male: Female ratio was 1.36:1. Of the 163 positive cases, minor clone (0.1–1%) was detected in 40 (24.5%), major clone in 95 (58.3%) and rare clones (0.01–0.1%) in 28 (17.2%). 28 cases showed clone size greater than 50%. PNH clone size in neutrophils, monocytes and RBCs ranged from 0.01 to 98.3%, 0.04–98.78% and 0.01 to 84.3% respectively. However, the median monocyte clone size (4.8%) was greater than the median neutrophil clone size (1.8%). Bone marrow was hypocellular in 87.7
Conclusions: PNH clone size less than 0.1

Tropical Sprue in Gastric Siderosis

Madhuchhanda Mallik, Tapaprakash Behera, Pallavi Bhuyan, Pranati Mohanty, Lity Mohanty

Introduction: Tropical sprue is a malabsorption syndrome characterized by chronic diarrhea, weight loss,and malabsorption of nutrients. It is thought to be infectious in etiology with a contributionof environmental factors. It affects the small intestine and is characterized by malabsorptionand multiple nutritional deficiencies, especially vitamin B12 and folic acid. The intestinalbiopsy findings show decrease in the villous height with villous atrophy. Gastric lesionsobserved in such patients includes simple gastritis as well as atrophic gastritis. Stainable iron has been found to be present in stomach and duodenum of patients with primary hemochromatosis and in individuals with alcohol abuse, which is a very unusual finding. We report here the presence of tropical sprue in a patient of secondary hemochromatosis who presented to us with chronic diarrhea.
Case report: A 16 year old femalepresentedwithchronicdiarrheaforthreemonths. She had been diagnosed with beta thalassemia major and was on regular bloodtransfusionssinceearlychildhood.Shehadbeenonchelationtherapybutwasnon-compliant.She had been taking folic acid tablets regularly. On clinical examination, she had pallor,glossitis and angular stomatitis. A complete blood count along with routine biochemicalinvestigationsweredone.Examinationofstoolrevealedsteatorrheaandpresenceofreducingsugar.Abdominalultrasonographyshowedhepatomegalywithmassive splenomegaly. Gastric biopsy showed iron deposition in gastric glands & macrophages. The tropical sprue was accounted for by the iron overload state of the patientandnon-adherencetochelationtherapy.
Conclusion: Tropical sprue has been found to occur in acute iron overdose, iron pill induced gastritis,hereditaryhemochromatosis,hepaticcirrhosisandrarely,inpatientswithahistoryofrepeatedbloodtransfusions.Depositionofironinthegastricmucosacauses tropical sprue. The finding of tropical sprue in thispatient in addition to the histopathological changes of gastric siderosis made the case even moreinteresting.

Alder-Reilly Anomaly In Hurler's Syndrome

Shivam Subudhi, Tapaprakash Behera, Pallavi Bhuyan, Pranati Mohanty, Lity Mohanty

Introduction: Hurler's syndrome, an autosomal recessive disorder of mucopolysaccharide metabolism caused by a deficiency of α-l-iduronidase enzyme manifests as abnormal granules in granulocytes.
Case report.

A 22-day-old male newborn baby presented with persistent neonatal jaundice since birth. On clinical examination, he had coarse facial features, a prominent forehead, an enlarged tongue, icterus, hepatosplenomegaly, skeletal deformities, and bilateral inguinal hernia. On investigation, peripheral smear revealed alder-reilly anomaly in the neutrophils suggesting mucopolysaccharidosis. Mucopolysaccharide excretion spot test of the urine was positive; and an assay for glycosaminoglycans in the urine was also high, which confirmed the clinical diagnosis of hurler's syndrome.
Conclusion: • Alder-Reilly anomaly in the granulocytes is a simple, easy, rapid diagnostic clue to hurler's syndrome.
• Bilateral inguinal hernia associated with hurler's syndrome is rare.

Extended Inflammatory Parameters In Hematology:The New Kid On The Block

Ginni Bharti, Tushar Sehgal, Imaculata Xess, Gagandeep Singh

Introduction: Activation status of white blood cells (WBCs) that are involved in sepsis can now be quantitatively evaluated thanks to a new set of haematological inflammation parameters that have been introduced. These parameters include neutrophils (NEUT-RI, NEUT-GI), immature granulocytes (IG), and activated lymphocytes (RE-LYMP, AS-LYMP). These measurements are what are known as extended inflammatory parameters (EIP). EIP has been very close to being employed in the assessment of these parameters in relation to bacterial and viral diseases, such as dengue fever, murine typhus, salmonellosis, leptospirosis, and malaria infections, as well as the COVID-19 infection. We evaluated these EIP in patients with parasitic, fungal and viral infections.
Aims & Objectives: 1. To evaluate EIP in patients with parasitic, fungal and viral infections.
2. To generate the threshold for EIP in patients with parasitic, fungal and viral infections.
Materials & Methods: This study was done in the Department of Laboratory Medicine at AIIMS New Delhi. In total, 100 participants were enrolled in the study: 20 patients each with parasitic, viral and fungal infection were included and 20 healthy persons were recruited in the control group. The haematological parameters were performed using the Sysmex XN automated analyzer. The following parameters were analyzed: Reactive Lymphocytes (RE-LYMP, expressed as an absolute number in [103/μL]), Antibody-Secreting Reactive Lymphocytes (AS-LYMP, expressed as an absolute number in [103/μL]), Neutrophil Reactive Intensity (NEUT-RI, expressed in [FI] units, describing the fluorescence intensity), Neutrophil Granularity Intensity (NEUT-GI, expressed in [SI] units, describing the light intensity of the scattered laser beam), White Blood Cells (WBC, [K/μL]), Red Blood Count (RBC, [M/μL]), Platelets (PLT, [K/μL]), Neutrophils (NEUT, [K/μL]), Lymphocytes (LYMP [K/μL]), Monocytes (MONO, [K/μL]), Immature Granulocytes (IG, K/μL], Red Blood Cell Distribution Width Standard Deviation (RDW-SD, [fl]), Mean Platelet Volume (MPV, [fl]), and Mean Cell Volume (MCV, [fl]).
Result: The mean value of EIP is different in various infections. Statistical analysis using STATA 14.0 would be done. The p value $< 0 >$

Haemophagocytic Lymphohistiocytosis And Antiphospholipid Syndrome In Postpartum Period: A Rare Case Report

Jyotirmoy Biswas, Roger Rathna,ChristopherDSouza,ArkadeepDhali

Introduction: Haemophagocyticlymphohistiocytosis (HLH) is a life-threatening condition of disproportionate immune activation, classified into primary and secondary types—the former presents in childhood whereas the latter is due to underlying predisposing conditions.
Aims & Objectives: To report a unique case of an overlap of secondary HLH and Antiphospholipid Antibody (APLA) syndrome.
Materials & Methods: A 25-year-old woman with parity of two presented on postoperative day 26 after lower-segment-caesarean section with fever and chills lasting 3 weeks. She was initiated on Artesunate-based combination therapy for suspected falciparum infection. Next day she presented to the emergency room with acute onset breathlessness, with tachycardia, hypotension, and hypoxia.
Result: The presentation along with elevated D-Dimers warranted a Computed-Tomography-Pulmonary-Angiogram, which revealed a minor pulmonary thromboembolism. Blood workup showed anaemia, thrombocytopenia and mildy elevated transaminases. Computed-tomography of abdomen showed hepatosplenomegaly, ascites and mesenteric lymphadenopathy. With this picture, infective and immunological causes were considered. Tropical fever workup and Antinuclear Antibodies were negative. APLA workup done as part of autoimmune coagulation profile revealed triple-positivity thereby provisionally diagnosed with APLA syndrome. Additional workup showed hypertriglyceridemia, hyperferritinemia, and hypofibrinogenemia. Bone-marrow biopsy was unremarkable. In accordance with 2004 guidelines, diagnosis of HLH was made. She was started with steroids.
and anticoagulants and showed clinical improvement. APLA was repeated after 12 weeks which confirmed the diagnosis.
Conclusions: In literature, secondary HLH has been reported to be associated with various autoimmune conditions, most notably Systemic lupus erythematosus, but never has there been any report of HLH with coexisting APLA, let alone describing the possibility of a causal relationship. The literature review discussed two conditions—(1)Catastrophic Antiphospholipid Syndrome (CAPS), characterised by multi-organ damage due to circulating APLA, usually seen in but not limited to patients with autoimmune diseases and, (2) Macrophage Activation Syndrome (MAS), where patients have an underlying autoimmune disease and then develop HLH. This patient fit into the diagnostic criteria for both CAPS and HLH—indicating their possible overlap. Since both their primary treatment modality is steroids, the management was not significantly affected, nonetheless it is important to understand the possibility and likelihood of their coexistence even if it is just for academic interest.

Role Of RDW CV In The Early Detection Of Iron Deficiency Anaemia In Pregnant Women

Donna Syngkli, Rashmi Deori

Introduction: Despite the advance health care system, iron deficiency anaemia still remains the most common type of nutritional deficiency in developing countries like India. So a rapid need to find a cost effective parameter is required for the early detection or screening of iron deficiency anemia in pregnant women in order to prevent maternal and foetus complications.
RDW CV is a parameter that can be advised along with complete blood count as it is cost effective, fast, easy to perform when all the other Rbc indices along with peripheral blood film are normal.
Aims & Objectives: To determine the role of RDW CV in the early detection of iron deficiency anaemia in pregnant women less than 20 weeks period of gestation by using hematologyanalyzer.
1 To compare RDW CV with iron deficient and non iron deficient.
2. To compare RDW CV with other Rbc indices.
3. To compare RDW CV with peripheral blood smear.
Materials & Methods: A hospital based cross sectional study in 120 pregnant women less than 20 weeks.
Result: The result of the study shows that RDW CV had a sensitivity of 82.3% and specificity of 97.4%.
Conclusions: Though iron profile is a gold standard test for detection of iron deficiency anaemia, RDW CV prove to be a useful and reliable marker for screening and early detection of iron deficiency anaemia in pregnant women.

Quality Evaluation Of Sample Collection Facilities (SCF) Of Resource Limited Medical Laboratories (RLML) In Various States Of India

Manikchandra Tiwari, Preeti Chavan, Vivek Bhat, Sumeet Gujral, PG Subramanian, Arti Rauthan

Introduction: Medical laboratory reports help clinicians in proper diagnosis and management of patients. Erroneous diagnosis due to lab reporting errors may lead to improper patient management and may lead to increased morbidity and mortality. Medical laboratory practice is divided into three different stages, pre-examination, examination and post-examination.
In India, National Board for Accreditation of Testing and Calibration Laboratories (NABL) has published a checklist for evaluation of sample collection facility (SCF) i.e., document NABL112. It is known that errors generated at pre-examination stage may account for up to 75% of total medical laboratory errors; 26% of these may have detrimental effects on patient care, as well as dissatisfaction with healthcare service. Considering this fact, we conducted a small study on various aspects in the pre-examination stage practice in resource limited medical laboratories (RLML) which caters to maximum population. Here we compared current practices at SCF of both accredited and non-accredited RLMLs with the recommendations in NABL112 checklist.
Aims & Objectives: Very less information is available on analysis of RLMLs following SCF checklist to improve their sample collection and handling practices and thus reduce occurring of such errors. The aim of this study is to find out the percentage SCFs complying to checklist criteria and to evaluate importance of accreditation as a tool of quality improvement.
Materials & Methods: SCFs of both accredited and non-accredited RLMLs was compared for compliance with criteria given in NABL112 SCF checklist. Among fifty SCFs, seven were from accredited and forty-three from non-accredited RLMLs. Compliance was assessed for forty-three criteria. Percentage SCFs complying to each criterion was evaluated; in addition, Chi-square testing with p-value significant at $p < 0.005$ was calculated for each criterion.
Result: All accredited SCFs showed compliance to most of the criteria. Out of forty-three criteria evaluated for both accredited and non-accredited SCFs, statistically significant difference in compliance was seen in thirty criteria with p-value < 0.005, whereas thirteen criteria showed non-significant difference.
Conclusions: Non-accredited SCFs were found to be in poor compliance as compared to accredited SCFs. There is scope of improvement for deficiencies noted at multiple levels at such SCFs. Pre-examination errors may be reduced by implementing criteria of related SCF checklist.

Binding Of Linezolid To Human Hemoglobin: Illustration Of Their Molecular Recognition By Spectroscopy, Calorimetry, And Molecular Modelling Techniques

Ayantika Paul, Payel Biswas, Sutithi Dey, Baishali Basak, Ipsita Chatterjee, Sanjay Kumar Paul, Rajen Haldar

Introduction: In recent years, the interaction dynamics between foreign substances and bio-macromolecules is getting more attention. Hemoglobin, a globular protein, has ample affinity to interact with soluble compounds present in its vicinity, and thereby may disturb the redox system. Linezolid, an oxazolidinone group of antibiotics was once considered as the reserved drug; however, it is now being used widely. It is found to interact with albumin; however, nothing is known regarding its interaction with hemoglobin.
Aims & Objectives: In this study, we have taken this opportunity to explore the interaction profile of linezolid and hemoglobin and its possible consequences.
Materials & Methods: Healthy human subjects (20 to 30 years) who were free from smoking habit, chronic diseases, and use of any tranquilizers, drugs, and anesthetics were selected for this study. About 2 ml blood was drawn by venipuncture, and hemoglobin was purified from washed erythrocytes using Sephadex G100. Linezolid I.V. Injection was purchased from Integrace private limited, the stock solution of linezolid was prepared in 0.01 M phosphate buffer (PB), pH 7.4. We studied the interaction of linezolid with human hemoglobin (Hb) using spectrophotometric, spectrofluorimetric, calorimetric, and molecular modelling techniques. The results were expressed as mean ± SD where applicable and P values < 0.05 was considered as significant.
Result: We observed that there was overall decrease in absorbances of hemoglobin in entire spectral range upon addition of linezolid. Gradual quenching of the tryptophan (Trp) fluorescence of hemoglobin upon addition of linezolid clearly indicates their interaction. The interaction is an entropy-driven spontaneous exothermic reaction and Hb has one binding site for linezolid as evidenced from isothermal titration calorimetry. Hydrophobic interaction and hydrogen bonding play major role in the linezolid-hb interaction as evidenced from molecular modelling study. The increased rate of co-oxidation and the decreased rate of esterase activity of hemoglobin indicate disruption of structural integrity of hemoglobin after interaction with linezolid.
Conclusions: Hemoglobin interacts with linezolid and the interaction perturbs its structural integrity as well as physio-chemical property.

Influence Of Cigarette Smoking Frequencies On Physico-Chemical Properties Of Erythrocyte

Payel Biswas, Rajen Haldar

Introduction: Cigarette smoking has reflected its adverse impact on various hematological parameters, which numerous researchers have pondered. However, most of their studies recruited a single group of smokers to determine the outcome. Thus, it is difficult to conclude the sole contribution of smoking in inducing the detrimental effects. A dose-dependent response pattern in terms of smoking frequency and mode of puffing should be considered prior to draw a final conclusion. Moreover, this pattern may also help in finding the extent of such toxicity.
Aims & Objectives: We tried to explore the grade of adverseness on the physico-chemical properties of erythrocytes with smoking habits in a dose-dependent manner, using various biochemical and biophysical techniques.
Materials & Methods: Twenty non-smokers, 10 light smokers, 10 moderate smokers, and 10 heavy smokers were recruited in this study. We examined morphology, intracellular ROS (IcROS), osmotic fragility, methemoglobin formation, membrane fluidity, cholesterol, triglyceride levels and oxidative stress markers of erythrocyte membrane. The difference in mean values of each group was compared using one-way ANOVA. The data were articulated as mean ± standard error of the mean (SEM) and P values of < 0.05 were considered significant.
Result: Spherocytes and stomatocytes were more dominant in smokers (mostly in heavy smokers). IcROS production, methemoglobin formation and membrane-bound hemoglobin concentration were significantly elevated with smoking gradations, which imprints a burden of oxidative insult inside the erythrocyte. An increase in protein carbonyl formation and lipid peroxidation, and a concomitant decrease in free thiol groups with smoking habits implicate oxidative modification of the erythrocyte membrane. Similarly, increased fragility and decreased fluidity of the membrane were also observed, which correlates to the augmented membrane cholesterol and triglyceride level.
Conclusions: Hence, from this study, we conclude that cigarette smoking dose-dependently perturbs the physico-chemical properties of erythrocytes.

Multimorbidity In PCOS Develops Structurally Modified Erythrocyte: A New Dimension To PCOS Research Spectrum

Sutithi Dey, Payel Biswas, Baishali Basak, Ipsita Chakroborty, Pratip Chakroborty, Rajen Haldar

Introduction: Polycystic ovarian syndrome (PCOS) is the most prevalent lifestyle disorder among women of reproductive age which dysregulates hypothalamus-pituitary-ovary (HPO) axis. Concomitant translation of metabolic dysfunctions along with anovulation and hyperandrogenism plays a major role in developing insulin resistance (IR), type 2 diabetes mellitus (T2DM), obesity in PCOS. Moreover, this disorder also conveys significant risk for poor cardiovascular health due to dyslipidemic condition.
Aims & Objectives: The gross distribution of elevated plasma lipids in PCOS may interplay with hemorheology and deformability of erythrocyte. This particular area remains unrecognized in case of PCOS research spectrum. So, our aim was to investigate the morphological and biochemical alterations among women with PCOS.
Materials & Methods: A case control study has been designed by selecting PCOS women (n = 10) satisfying Rotterdam criteria (under no treatment), and age-matched healthy controls (n = 10). Erythrocytes were examined using scanning electron microscope. We studied erythrocyte membrane cholesterol content along with its fragility and fluidity. Intracellular ROS (IcROS) generation, estimation of oxidative stress markers of erythrocyte membrane was also performed in each group. For statistical analysis difference in mean values were compared using t-test. The data were represented as mean ± standard error of the mean and P values of < 0.05 were considered significant.
Result: We observed significant morphological alterations in PCOS women with occurrence of increased amount of spherocytes, stomatocytes and echinocytes under scanning electron microscope. PCO women showed significantly elevated clinical dyslipidemic profile along with an increased erythrocyte membrane cholesterol level. These cells further show significant decrease in erythrocyte membrane fluidity with surprising decrease in the magnitude of osmotic fragility. Moreover, elevation in IcROS level with concurrent increase in lipid peroxidation, protein carbonylation of erythrocyte membrane and reduction in free thiol group confirm generation of oxidative stress in erythrocyte of PCOS women.
Conclusions: Thus, we conclude that dyslipidemic condition in women with PCOS develops structurally modified erythrocyte and generates oxidative stress within the erythrocyte.

Evaluation Of Erythrocyte's Oxidative Status Along With Its Architecture In Iron Deficiency Anemic Women Of Reproductive Age

Baishali Basak, Payel Biswas,Tuphan Kanti Dolai,Rajen Haldar

Introduction: Iron deficiency anemia (IDA) has attained epidemic extents in developing countries and has become a crucial public health issue worldwide. Women of reproductive age hold the peak position in this instance. The disproportion between iron intake, absorption, storage and iron utilization or loss concludes into IDA. The inadequate synthesis of hemoglobin due to less amount of iron results in altered morphology of erythrocyte. Although few is known about the architectural alteration in human erythrocyte, however, nothing is known about the hemoglobin in IDA subjects.
Aims & Objectives: The aim of the study is to explore the oxidative status along with erythrocyte architecture in IDA women of reproductive age; and the objectives are: (i) study of the alteration of erythrocyte architecture (if any) and (ii) study of physico-chemical properties of hemoglobin.
Materials & Methods: Age-matched 12 healthy women and 12 IDA women were recruited in this study. About 2 ml of venous blood were taken to carry out the desired experiments. We studied the architecture of erythrocyte, membrane-bound hemoglobin, carbonyl content and free thiol groups of erythrocyte membrane. We also measured free plasma hemoglobin, co-oxidation, carbonyl content, esterase-like activity and ferryl formation of hemoglobin. The statistics were done using student's t-test. The data were expressed as mean ± standard error of the mean (SEM) and P values of < 0.05 were considered as significant.
Result: The formation of echinocytes and elliptocytes was increased in IDA women. There were increased in membrane-bound hemoglobin, carbonyl content and decrease in thiol groups of erythrocyte membrane in women with IDA. Furthermore, the biochemical and biophysical parameters of hemoglobin showed that there were increase in free plasma hemoglobin, rate of methemoglobin formation, carbonyl content, esterase-like activity and ferryl formation in IDA women. These finding clearly indicate the oxidative damage of erythrocyte along with its structural alteration and the altered physico-chemical properties of hemoglobin in IDA women.
Conclusions: This study indicates that the elevated oxidative damage of erythrocyte which may be the reason behind the modification of physico-chemical property of hemoglobin along with the alteration of architecture of erythrocyte in IDA women of reproductive age.

Detection Of Macrothrombocytopenia Using Flow Cytometry: A Novel Technique

Sunistha Bhattacharjee, Suryyani Deb, Sandeep Saha, Namrata Dhara, Maitreyee Bhattacharyya

Introduction: Inherited macrothrombocytopenia is the most frequent form of inherited thrombocytopenia, represents a heterogeneous group of disorders characterized by a reduction in the number (platelet count) and increase of platelet size (MPV). A large group of individuals with this condition may never have clinically significant bleeding but often fails to be diagnosed by complete blood count (CBC), as in most of these cases MPV falls out of the range (> 12). Therefore determination of macrothrombocytopenia through an easy technique/protocol is the need of the hour.
Aims & Objectives: The aim of this research was to develop a flow cytometry based technique/protocol to determine macrothrombocytopenia.
Materials & Methods: Healthy blood samples has been collected from thalassemia screening camps. Blood has been drawn with informed consent. CBC was done using Sysmex XN-1000. Peripheral blood smear was prepared and analysed under microscope. For the platelet Flow cytometry (BD FACS Canto II) citrated blood was used. Platelets were detected by platelet specific marker CD41a (conjugated with PE-Cy7). A protocol was developed based on high forward and side scattering (FSC and SSC) to determine bigger sized platelets. The results were then cross verified with the microscopic data of the corresponding samples.
Result: 210 healthy samples were taken for initial detection for macrothrombocytopenia by hemogram analyser and peripheral blood smear. Further 20 samples were taken for this pilot study and platelet size was measured using a gating strategy to identify CD41a + platelets. A dot plot has been made with side scatter (SSC) vs. forward scatter (FSC) plot. It was observed that 9 individuals having lower number of platelets ($< 180 >$
Conclusions: Therefore, it can be said that the developed protocol can effectively determine bigger sized platelets and may be used as an alternative for better diagnosis of macrothrombocytopenia.

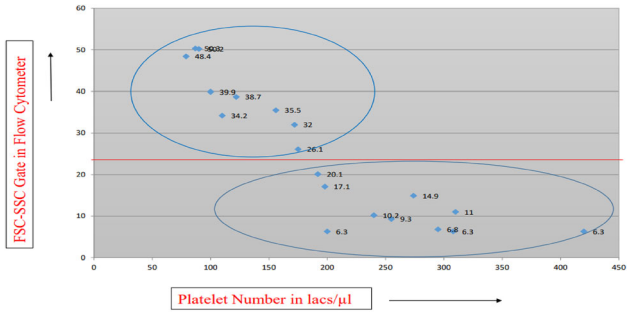


Figure :- Platelet Number vs FSC-SSC

Clinicopathological And Etiologicalprofile Of Pancytopenia In Pediatric Patients In A Tertiary Care Institute Of Eastern India

Parni Verma, Ruchi Sinha, Surabhi, Tarun Kumar, Shreekant Bharti, Punam Prasad Bhadani

Introduction: Pancytopenia refers to reduction in all the three peripheral blood cell lineages below the normal reference ranges according to age and sex. It is a manifestation of an underlying disease process. Pediatric pancytopenia could be due to congenital or acquired causes. Early recognition of its etiology will have impact on treatment of these vulnerable pediatric patients.
Aims & Objectives: To study the clinical features and etiology of pancytopenia in pediatric population in a tertiary care institute of Bihar.
Materials & Methods: An observational hospital-based study was conducted on pancytopenicpediatric patients in AIIMS Patna from July 2020 to August 2022. A detailed clinical history, blood investigations, bone marrow examination and biochemical analysis were done in all patients. WHO defines pancytopenia as hemoglobin $<$ 10 g/dL, absolute neutrophil count $< 1.5 \times 10^9$/L and platelet count $< 100 >$ 9/L.
Result: Out of 75 patients, 62.6% (n = 47) were males and 37.3% (n = 28) were females. Majority of patients 73.3% (n = 55) were between the age group of 7-14yrs. Fever was the most common presenting complain in 82.6%(n = 62). Lymphadenopathy, hepatomegaly and splenomegaly was seen in 9.3%(n = 7), 17.3% (n = 13) and 8%(n = 6) cases respectively. CBC demonstrated severe anemia (hemoglobin $<$ 8 g n = 39), $>$ 9 /L) in 30.6%(n = 23) and platelet count $< 50 >$ 9/L in 33.3%(n = 25) cases respectively. Biopsy revealed a hypocellular marrow in 44% (n = 33) and hypercellular in 5.3% (n = 4). Aplastic anemia was the most common cause in 29.3% (n = 22) followed by hematological malignancy in 21.3%(n = 16) cases. Very severe aplastic anemia was present in 14.5%(n = 11) cases. PNH clone was identified in 3 of 22 cases of

aplastic anemia (4%). Other causes included nutritional deficiency (9.3%), HLH (2.6%), parvovirus infection (1.3%), hepatitis C infection (1.3%), autoimmune (1.3%) and down syndrome (1.3%).
Conclusions: Aplastic anemia was found to be the most common cause of pancytopenia followed by hematological malignancy in children in our study. Hence bone marrow examination, flow cytometry and biochemical analysis along with routine blood investigation play an important role to approach cases of pancytopenia.

Thromboelastometry (ROTEM): Normal Reference Ranges And Correlation With Standard Coagulation Tests In Healthy Indian Adults

Ankita, Anjali Sharma, Shruti, Mukul Singh, Sunil Ranga

Introduction: Viscoelastic coagulation tests like Rotational Thromboelastometry(ROTEM) and Thromboelastography (TEG) are used in complete evaluation of clot initiation, formation and stability as compared with traditional laboratory methods.
Aims & Objectives: The aim of this study was to determine normal reference ranges for ROTEM and to compare standard coagulation parameters (Prothrombin Time(PT), Activated Partial Thromboplastin Time(APTT) and Fibrinogen levels) with ROTEM values in healthy adult population.
Materials & Methods: This observational study was conducted in the Department of Pathology, VMMC & Safdarjung Hospital, New Delhi. Total 40 healthy subjects were included. Citrated blood samples were taken for standard coagulation testing and ROTEM respectively and their values were compared as follows: (i) CT and Clot formation Time(CFT) of extrinsically activated ROTEM assay with PT; (ii) CT and Clot formation Time (CFT) of intrinsically activated ROTEM assay with APTT, and (iii) comparison of standard Fibrinogen test to be compared with clot firmness after 10 min (FIBTEM A10) and to the maximum clot firmness (FIBTEM MCF) of the FIBTEM Assay. FIBTEM test was based on an extrinsically activated ROTEM assay that also contains the platelet inhibitor to separately evaluate functional fibrin polymerization without platelet activity.
Result: On data analysis, the ROTEM reference ranges in healthy adults are derived statistically. However, the sample size is too less but the results are comparable to western adult population. On comparing the ROTEM values and standard coagulation parameters in healthy adults, it was found that PT and APTT cannot be used interchangeably with the ROTEM values.
Conclusions: There is no single laboratory test which is capable of comprehending all aspects of blood coagulation and fibrinolysis. Further large studies are required to evaluate and standardize ROTEM technology, in combination with standard coagulation tests.

Hemophagocytic Lymphohistiocytosis: Experience At A Tertiary Care Centre

Namrata Kaul, Rajesh Kashyap, Ruchi Gupta, Khaliqur Rahman, Dinesh Chandra, Manish Kumar Singh

Introduction: Hemophagocytic lymphohistiocytosis (HLH), is a life-threatening hyperinflammatory condition characterized by immune dysregulation, cytokine storm, unfavourable outcome. It can be primary (genetic) or secondary to infections, neoplasms and other inflammatory conditions.
Aims & Objectives: To evaluate the clinico-pathological features and outcome in patients of HLH, and critically analyze the available diagnostic criterias.
Materials & Methods: In this a single centre retrospective study, all cases of HLH diagnosed and treated from January 2019 to June 2022 were reviewed. The clinical information and laboratory parameters were retrieved from electronic medical records. The diagnosis of HLH was made as per the HLH 2004 protocol and H score. NK cell activity and soluble CD25 levels mentioned in the HLH 2004 protocol were not included as these parameters were not done at our centre. Therefore, only patients who fulfilled the remaining five out of six criteria were included.
Result: Sixty seven patients presented with clinical features of HLH and showed hemophagocytosis in the bone marrow. Out of these 67 cases, 46 and 51 fulfilled the diagnostic criteria, according to HLH 2004 protocol and H score respectively. There was no sex predilection. 30 adult patients and 16 pediatrics patients were seen. The median age of presentation in adults and children was 43 years and 6 years respectively. We had only one genetically proven primary HLH, associated with Type 2 Griselli syndrome. Rest all were secondary. Infection was the commonest cause of secondary HLH followed by autoimmune disorders. We had two malignancy associated and one post allogenic stem cell transplant associated. All patients had one or the other cytopenia. 32% patients had increased triglyceride levels. Reduced fibrinogen was seen in 22% patients. Ferritin and AST was elevated in all. Among these 46 patients, 24 were successfully treated and 18 patients died.
Conclusions: HLH may remain under diagnosed with HLH 2004 diagnostic criteria as it includes molecular biomarkers. Persistent fever not responding to antibiotics alongwithcytopenias and splenomegaly should a raise suspicion. It is important to distinguish primary from secondary HLH as treatment of the underlying disorder is also essential in the latter. Early diagnosis and prompt treatment helps in enhanced outcome.

Erythrocyte Develops Natural Defense Against Oxidative Imbalance In Cigarette Smokers

Rajen Haldar, Jyotirmoy Sikdar

Introduction: The free radicals and different reactive species emitted from a lit cigarette generates oxidative impairment to the circulating erythrocytes. The possible cellular damage is compensated by its antioxidant defense systems including enzymatic and non-enzymatic pathways. Though, there has been a contradictory aspect that the increased production of reactive oxygen species in smokers may exceed the capacity of the antioxidant defense system, and decrease in overall protective efficacy.
Aim and Objectives: In such an imbalance ambiance, Glut1, which transports glucose and dehydroascorbate to erythrocytes, was selectively chosen to tackle the oxidative insult by scavenging reactive oxygen species (ROS) amongsmokers. To obtain the hypothesis, we explored some hidden facts behind erythrocyte modifications withemphasis on its membrane proteins by different biochemical and biophysical techniques.
Materials and Methods: Twenty smokers and 20 non-smokers, of 25 to 35 years among healthy male volunteers were recruited in this study. We examined morphology, intracellular ROS and osmotic fragility and fluidity of erythrocytes. The defensive property and characterization of Glut 1 were portrayed by gel electrophoresis, immunoblot and immunohistochemistry technique, glucose consumption and flow cytometric assays.
Results: We observed significant increase in stomatocytes and spherocytes, and osmotic fragility of erythrocyte, along with reduced level of protein thiol and decreased membrane fluidity. Denaturing gel electrophoresis indicated alteration in Glut 1 (i.e., band 4.5). The increased Glut 1 level in smokers was confirmed by immunoblotting and immunocytochemistry. Furthermore, smokers showed significantly higher glucose uptake in whole blood. The intracellular ROS was significantly higher in smokers as evidenced by flowcytometric assay. Glucose and DHA alone or together significantly reduced

IcROS at higher rate in smokers. However, in presence of Glut 1 specific blocker, phloretin, neither glucose nor DHA could reduce IcROS in both non-smokers and smokers. This confirms that Glut 1 by transporting glucose or DHA attenuates IcROS.
Conclusion: Thus, we conclude that the erythrocytes of cigarette smokers having altered morphology though developed a defense system by upregulating Glut 1 to combat with enhanced intracellular oxidative insult.

Bone Marrow Necrosis In Trephine Biopsies: A 5 Years Study In A Single Tertiary Care Centre In Eastern India

Shreshtha Talukde, Sarita Pradhan, Rajesh Kumar Bhola, Priyanka Samal, Debahuti Mohapatra

Introduction: Bone marrow necrosis is a relatively uncommon entity encountered in trephine biopsies with an incidence of 0.3–2%.Theetiology ranges from non neoplastic to neoplastic conditions with the latter being most common. Clinical findings associated with bone marrow necrosis include bone pain, fever, elevated LDH, cytopenias and leukoerythroblastic blood picture.
Aims & Objectives: To assess the frequency and determine the etiology of myelonecrosis.
Materials and methods: This was a retrospective study carried out in a tertiary care setup in eastern India from.
September 2017 to August 2022.All bone marrow biopsies with necrosis were retrieved and categorized into grades based on the extent of necrosis seen in the trephine biopsy. Percentage marrow necrosis was defined as the percentage of total marrow cellularity and was divided into.

mild($< 20\%$),moderate (20–50%) and severe($> 50\%$).Etiology was determined as per the trephine biopsy findings. Wherever feasible, ancillary techniques like immunohistochemistry,special stains and flowcytometry were used.
Results: Out of a total of 4255 cases, 31 cases showed bone marrow necrosis (0.7%). The age of presentation ranged from 7 to 84 years. Out of all cases,18 cases (58%) presented with cytopenias. Normal CBC and peripheral smear was seen in 9 (3%)cases which showed less than 20% marrow necrosis. Bone marrow aspiration was difficult and hemodilute in 55% cases and was inconclusive in 6% cases. The most common underlying causes were haematopoietic malignancies in 35.5% cases and non-haematopoietic malignancies in another 35.5% cases.
ALL(7 cases) and NHL(4 cases) were the common haematopoietic malignancies in our study. Non-neoplastic causes included sickle cell disease (1 case), sepsis(1 case) and tubercular granulomas (7 cases).
Conclusion: Bone marrow necrosis is encountered infrequently in routine practice and may pose diagnostic difficulties in bone marrow aspiration smears. Underlying causes are often determined intrephine biopsy and may need ancillary techniques like immunohistochemistry and special stains for definite diagnosis.

A Study On Biochemical And Haematological Profile With Reference To Reticulocyte Haemoglobin Content (Ret-He) In Differentiating Iron Deficiency, Iron Deficiency Anemia And Non-Iron Deficiency Anemia In Patients Of Chronic Kidney Disease

Anita Kumari Sahu, Pramod Kumar Tudu, Udaybhanu Rout, Priyanka Samal

Introduction: All the clinicians must not overlook the fact that patients with chronic kidney disease (CKD) may have iron deficiency anemia (IDA).Anemia in CKD is associated with reduced quality of life,increased incidence of cardiovascular disease,hospitalization and mortality. Therefore, the early detection of IDA can enhance the efficiency of management and quality of life for CKD patients. The aim of this study was to assess the role of reticulocyte hemoglobin (Ret-He) in differentiating various types of anemia in CKD patients.
Aims & Objectives: The aim of this study was to assess the role of reticulocyte hemoglobin (Ret-He) in differentiating various types of anemia in CKD patients.
Materials & Methods: A prospective observational study was conducted among 150 CKD inpatients in our hospital. The study population was divided into 3 groups—CKD with ID (50), CKD with IDA (50) and CKD having anemia without Iron deficiency (50). The study was done from April till September 2022. ANOVA and Receiver operator characteristics (ROC) curves and the area under the curve (AUC) models were used to evaluate variations in Ret -He against Serum Ferritin, transferrin saturation and Serum Iron.
Result: The RET-He was significantly lower in the IDA group ($\mu = 20.70 + 1.86$) compared to other groups (ID: $\mu = 28.31 + 3.7$, Non-ID Anemia $\mu = 31.6 + 3.7$, $p < 0.05$). There is a positive correlation between Ret-He and different types of anemia in CKD patients. Ret-He was positively correlated with Ferritin level, Hb, Transferrin saturation and serum Iron level ($p < 0.005$) but negatively correlated with TIBC and platelet count($p > 0.05$). Ret-He (pg) values of < 23.5, 23.5–24.5 and > 24.5 were highly sensitive and specific for iron deficiency anemia, ID group and Non-IDanemia group respectively.
Conclusions: RET-He, TSAT and ferritin can predict the iron deficient status in CKD patients.

Point Of Care Diagnostic Tools For Screening Of Sickle Cell Disease

Ravindra Kumar, Aparup Das

Introduction: Diagnosis of disease is the prime factor for the prevention and control and same is true for Sickle cell disease. Sickle cell disease is highly prevalent in tribal population of India. Tribes are the most marginalized communities and mostly live in hard-to-reach remote hilly and forested areas. Therefore, diagnosis of sickle cell disease in these far-flung areas through high end diagnostic method such as cation exchange-high performance liquid chromatography, capillary electrophoresis and cellulose acetate gel hemoglobin electrophoresis is most challenging. Therefore, there is an urgent need for the point-of-care devices for door-step screening of sickle cell disease in a highly prevalent and hard-to reach remote areas.
Aims & Objectives: The aim of study is to review the available point-of-care devices for the screening of sickle cell disease.
Materials & Methods: Literature regarding point-of-care devices for the screening of sickle cell disease was searched on different databases such as PubMed, Scopus, Web of Science and Google Scholar. Sensitivity and Specificity of each devices were recorded and compared.
Result: There are three point-of-care devices currently available for the diagnosis of sickle cell disease.
1. Sickle SCAN is a rapid diagnostic test based on direct lateral flow chromatographic qualitative immunoassay technique to detect the presence or absence of HbA, HbS and HbC. Hemoglobin variants specific polyclonal.

antibodies are used for the detection of specific hemoglobin variant. It has 100% sensitivity and specificity for the SCD whereas it is 100% sensitive and 98% specific for diagnosis of sickle cell trait. The assay is also suitable for new born screening due to its ability to identify the HbS even in presence of high HbF (upto 96%).

3. HemoTypeSC is another rapid diagnostic test based on competitive lateral flow immunoassay. This rapid diagnostic kit has hemoglobin variant specific monoclonal antibodies. It can differentiate the sickle cell trait with sickle cell disease and also suitable for

newborn screening for sickle cell disease. HemoTypeSC had an overall sensitivity of 99.5% and specificity of 99.9%

Conclusions: All the point-of-care devices mentioned above have high sensitivity and specificity and able to differentiate the sickle cell trait and sickle cell disease. These point-of-care devices should be preferred over solubility and sickle slide test as it may differentiate the sickle cell trait and sickle cell disease which can be beneficial for mass screening for sickle cell disease.

Inflammatory Markers In Acute Myeloid Leukemia Before And After Therapy: A Comparative Study

Ajit Singh, Sumit Dokwal

Introduction: Several inflammatory markers are involved in etiopathogenesis of acute myeloid leukemia. Out of them, TNF is a major regulatory cytokine which stimulates proliferation of dividing cells while inducing apoptosis in mature progeny. B2M, a known prognostic factor in multiple myeloma and reflects tumor burden and turnover. While in vitro studies have shown conflicting effects of TNF on AML cells few clinical studies have shown TNF and B2M to have prognostic relevance in AML.

Aims & Objectives: To assess the status of TNF and B2M in patients of AML before and after chemotherapy.

Materials & Methods: 15 newly diagnosed cases of AML & 15 age and sex matched healthy controls were taken. AML patients were treated with a combination chemotherapy of cytarabine and an anthracycline. Routine biochemistry, complete hemogram, serum TNF and B2M tests were performed in newly diagnosed patients before treatment and in controls. The investigations were repeated at first complete remission or after 4 weeks of chemotherapy (whichever is earlier). TNF and B2M were measured by ELISA.

Result: 60% patients achieved remission, 26.7% did not achieve remission and 13.3% expired. Baseline TNF and B2M levels were significantly raised in AML patients (TNF:7.32 $\pm$ 23.24 pg/ml vs not-detectable, B2M:1.76 $\pm$ 0.95 µg/mLvs 0.99 $\pm$ 0.67 µg/mL, $p = 0.004$). B2M levels at diagnosis were significantly higher in patients who expired than patients who achieved remission ($p = 0.034$). B2M levels were significantly correlated with known prognostic factors;total leucocyte count and blast count in peripheral blood($p = 0.001$ & 0.001). TNF and B2M levels decreased significantly after chemotherapy only in remission group ($p = 0.015$).

Conclusions: Serum TNF and B2M levels have prognostic value in AML patients. Further studies in larger number of patients with long term follow up are required to validate these findings.

Synchronous Occurrence Of Multiple Myeloma And Chronic Myeloid Leukemia With A Common Cell Of Origin

Pranay Gurung, Akshaya Mandloi, Guntiboina Vinay Anand, Aaishwarya Dhabe, Gorantla Ashish Babu, Samipa Das, Sushant Vinakar, Asish Rath, Arijit Nag, Saurabh Jayant Bhave, Jeevan Kumar, Reena Nair, Deepak K Mishra, Mayur Parihar

Introduction: The co-occurrence of Chronic Myeloid Leukemia (CML) and Multiple Myeloma (MM) is extremely rare with only seven cases reported till date. We present a case of synchronous CML and MM investigating common clonal origin of the two malignancies using Fluorescentin situ hybridization (FISH).

Aims & Objectives: To investigate the common clonal origin in a patient with synchronous co-occurrence of CML and MM.

Materials & Methods: FISH was performed using myeloma panel on CD138 enriched plasma cells: *IGH* break-apart probe, *TP53/CEN17* deletion probe, *CDKN2C/CKS1B, CEN 5/9/15* and *BCR::ABL1* fusions probes following standard techniques.

Result: A 72 yr old patient came with complaints of bowel and bladder incontinence. A complete blood count revealed leukocytosis (98.4 $\times$ 109 /L), thrombocytosis (670 $\times$ 109 /L) and anemia (Hemoglobin:100 gm/L) with 06% blasts. Peripheral blood and bone marrow (BM) aspirate examination was consistent with the diagnosis of CML in chronic phase (CML-CP). The BM aspirate showed 5% plasma cells as well. FISH was positive for *BCR::ABL1* fusion.

The BM biopsy revealed features of CML-CP in few intramedullary spaces and sheets of clonal plasma cells in other intramedullary spaces highlighted by CD138 and CD56 with kappa restriction. The biopsy of the rib lytic lesion showed a Plasmacytoma. Free light chain assay showed increased Kappa light chain (181.11), with Free Kappa/Lambda ratio of 26.21 and IgG Kappa light chain restriction on immunofixaiton electrophoresis. Myeloma FISH panel on CD138 positive cells was positive for 1q amplification 40% of cells.

To investigate the clonal origin of the two malignancies, FISH for BCR::ABL1 was performed on CD138 positive cells (plasma cells) and *BCR::ABL1* fusions were seen in 65% of cells. Further analysis by co-hybridizing *BCR::ABL1* fusion probe and 1q probe on CD138 positive cells revealed presence of both *BCR::ABL1* fusions and 1q amplification in same cells suggesting a common clonal origin for CML and MM in our patient.

Conclusions: Pluripotent progenitor stem cell can differentiate into both lymphoid and myeloid lines and result in synchronous co-occurrence of CML and MM as was seen in our patient.

Examination Of Bone Marrow Microenvironment: Technical And Interpretative Challenges

Sreerag K, James Devasia, Rakhee Kar, Rajesh NG, Biswajit Dubashi, Debdatta Basu

Introduction: Bone marrow microenvironment is a supportive system composed of several different cell types, cytokines, and matrix proteins that are essential for the proliferation and differentiation of hematopoietic stem and progenitor cells. Angiogenesis is a hallmark of cancer cells and helps in sustaining and growth of tumors including leukemia and studied by measuring mean vascular density (MVD). Immunohistochemistry (IHC) on bone marrow is used to study the microenvironment. Standardization of IHC staining protocol is the key to achieving a satisfactory staining result as it verifies analytical components of the test.

Aims & Objectives: 1. To standardize antigen retrieval medium and pH, primary antibody concentration, staining time, and temperature for immunohistochemistry for Osteopontin, FOXP3, SDF1/CXCL12, CD44, N-cadherin, IL-7, and CD150.

2. To compare the MVD by manual method with digital pathology Image analysis (DPIA) tool in bone marrow

Materials & Methods: Processing of tissue specimens on APES-coated glass slides was done according to laboratory protocol. The antigen retrieval was done by pressure cooker with citrate as well as Tris–EDTA buffer. Immunostaining was done with different concentrations of primary antibodies for specified time and temperature. Concentration of antibody at which least background staining and optimal antigenic staining was considered satisfactory. All the slides were examined by two pathologists, blinded for the dilutions.

CD34 stained bone marrow biopsies of 37 patients with CML were studied for MVD. The regions of interest (ROI) were selected at 10 $\times$ magnification and the snapshot of the ROI was taken at 40 $\times$ from the whole slide image. This ROI was used for manual counting by an experienced pathologist and DPIA. Images were analyzed using the OpenCV library. Areas less than 500 pixels were eliminated.

Result: The result of standardization is summarized in table 1. The mean (SD) of MVD for manual 11.5 (5.4) was per image, while for

the DPIA was 12.3 (6.4). Both methods gave a good correlation ($p < 0.001$, Rho = 0.825).
Conclusions: The standard operating procedure for IHC for the bone marrow microenvironment was established. The standardization process, although cumbersome, if done meticulously, helps in achieving optimal staining results in immunohistochemistry. Calculation of MVD by DPIA with OpenCV is a viable alternative to the time-consuming manual method.

Evaluation Of Tyrosine Kinase Resistant Mutations And Their Clinical Relevance In Indian CML Patients

Suman Lata, Hare Ram Pandey, Seema Tyagi, Manoranjan Mahapatra, Tulika Seth

Introduction: Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm, characterized by translocation t(9; 22)(q34; q11) resulting in BCR-ABL1 fusion protein with high tyrosine kinase activity. Since 2001, management of CML was revolutionized by discovery of 1st generation Tyrosine kinase inhibitors (Imatinib Mesylate) which has been used as front-line targeted therapy.
Aims & Objectives: Over the last decade, the treatment of chronic myeloid leukemia has progressed tremendously. 20% of the CML patients show resistance to Ist generation TKI. The aim of study was to find out the relevance of finding acquired KD mutation early and see the Imatinib treatment response. It may cause resistance to second-line therapy as it may give rise to very aggressive multi-mutated clones. This study has been introduced to understand the significance of progression to AP/BC in CML, in patients who are positive for ABL1 KD mutations.
Materials & Methods: Screening for acquired TKD mutation in CML patients was performed in who failed imatinib carried out by DNA sequencing for analysis of mutations of the BCR-ABL kinase domain and the association between disease characteristics and outcome variables such as mutation status and survival were evaluated and correlated with treatment.
Result: We have observed significant association with new tyrosine kinase inhibitors (TKI) nilotinib and dasatinib became available during the follow-up of these patient populations. BCR-ABL mutations can confer high-level resistance to TKIs and promote the disease progression from chronic phase to accelerated phase (AP) and blast crisis (BC).
Conclusions: Our findings have concluded that the mutations occur frequently among patients who develop resistance to imatinib, and mutations reported to be most insensitive to imatinib (e.g., T315I) were found infrequently in this series. It has been reported that not all mutation have the same level of resistance to Imatinib or other agents intensity and the management after failure with imatinib as many strategies are emerging that may overcome resistance to imatinib.

Anterior Myeloid Sarcoma With Acute Myeloid Leukemia: An Unusual Case

Vinita Paswan, Khaliqur Rahman,Neeraj Kumari,Zafar Neyaz

Introduction: Myeloid sarcoma(MS) is a rare disease entity that can present as an isolated extramedullary tumor (EM) of immature granulocytic cells. Although myeloid sarcomas (MS) are frequently associated with acute myeloid leukemia (AML), the occurrence of mediastinal MS is a much rarer event.
Aims & Objectives: Here we report a case of mediastinal myeloid sarcoma seen in association with acute myeloid leukemia (AML).
Materials & Methods: A 33-year-old male presented with a complaint of chest pain, dyspnea, and fever without organomegaly or lymphadenopathy. Peripheral blood, Computed tomography-guided biopsy, and bone marrow examination was performed. Immunohistochemistry and Immunophenotyping were performed on mediastinal mass and bone marrow aspirate samples respectively.
Result: Computed tomography thorax demonstrated an anterior mediastinal mass which on histopathological examination revealed malignant infiltration by medium to large-sized atypical cells, which were positive for LCA, TdT, CD4, and CD117; while being negative for cytokeratin, PAX5, CD3, and CD20. A complete hemogram revealed leukocytosis with 32% blasts. Bone marrow aspirate followed by flow cytometry was diagnostic of acute myeloid leukemia. A final diagnosis of Acute Myeloid Leukemia with mediastinal myeloid sarcoma was put forth. The patient was planned for standard AML-directed 3 + 7 induction chemotherapy. However, the patient rapidly deteriorated and succumbed within a week of diagnosis owing to superior vena cava syndrome.
Conclusions: Despite the rarity of mediastinal mass localization, clinicians must have a high index of suspicion of mediastinal myeloid sarcoma to ensure timely therapeutic intervention results in significantly prolonged leukemia-free survival.

Primary Ovarian hemangioma- A rare, incidental finding

Shailasuta Das, Prashanta Kumar Das, Sushruta Mohanty

Background: Vascular tumors of female genital tract especially those of ovary are very rare. Cavernous hemangiomas of ovary are rare and are incidentally detected during surgery or autopsy. The exact incidence is not mentioned in the literature and available information is only through case reports.
Case summary: A 55 year old female complaining of severe abdominal pain was admitted to gyneacology ward. USG revealed a left ovarian cystic mass measuring 4 cm diameter. Serum tumor markers were normal. Hysterectomy was done and sample was sent for histopathological analysis.
Grossly, we received uterus, cervix measuring 8 × 6x5cm with attached ovaries and tubes. Cervix grossly appeared normal. Lower uterine segment showed an intramural mass measuring 4 cm diameter. Cut section of the left ovary appeared spongy greyish-black with multiple hemorrhagic spots. Other side ovary grossly appeared normal.

Microscopically, a small vascular lesion was incidentally diagnosed that showed numerous various sized thin walled vascular channels lined by single layer of endothelial cells separated by connective tissue septa. No atypia, necrosis or mitotic figures noted. Immunohistochemistry revealed that CD34 was strongly positive for the lining that confirms the vascular nature of the lesion.

Finally, the diagnosis of primary ovarian hemangioma (cavernous type) was rendered.
Conclusion: Ovarian hemangioma are rare, benign vascular tumors occurring in a wide age range and should be considered in differential diagnosis when a growing adnexal mass or hemorrhagic ovarian lesion is suspected in post menopausal women.

Serum tryptase as an adjuvant biomarker in chronic phase chronic myeloid leukemia

Anisha Mathew, Manisha Naithani, Sarama Saha, Rituparna Chetia, Arkapal Bandyopadhyay, Sudeep Vaniyath, Debranjani Chattopadhyay, Anamika Bakliwal, Ashok Rajoreya, Uttam Kumar Nath

Introduction: CML has been prognosticated using clinical scores such as the European Treatment and Outcome Study (EUTOS), SOKAL, and the EUTOS long term survival score (ELTS) which use basophil percentage or blast count following the discovery and use of

Tyrosine Kinase Inhibitors. However, the total basophil and blast compartment in CML may often exceed the morphologically identifiable fraction of basophils, hence presenting an unmet need for novel adjuvant biomarkers. Thus, this study focuses on Tryptase as a possible adjuvant prognostic biomarker.
Aims & Objectives: To study role of serum Tryptase as a prognostic biomarker in chronic phase Chronic Myeloid Leukemia.
Materials & Methods: Longitudinal study with a total of 40 newly diagnosed patients (convenience sampling) yet to be started on treatment. Baseline blood basophil percentage and serum tryptase levels were measured in all patients at the time of diagnosis, with hazard scoring calculated using SOKAL, EUTOS, and EUTOS Long Term Score (ELTS) score. Quantitative PCR (index test) for BCR-ABL1 transcript level estimated at baseline and after 3 months of imatinib therapy to establish diagnosis and assess early molecular response.
Result: Significant correlation ($p < 0.05$) was seen between EUTOS score and ELTS score and serum tryptase levels. Serum tryptase in linear regression studies showed significance to predict molecular response even after adjustment with other parameters like peripheral basophil levels, EUTOS, and SOKAL scores ($p < 0.05$). The ROC curve was plotted for serum tryptase which had an area under the curve (AUC) of 0.74 (P = 0.002, 95% CI: 0.649 60 0.939) which was higher than the AUC for EUTOS, SOKAL, and ELTS score ($P > 0.05$).
Conclusions: This study indicates that serum tryptase can have a potential role as an adjuvant prognostic biomarker in CML-CP patients.

Myeloid Sarcoma: A rare case presentation

Gargi Kapatia, Akriti Jindal, Dhwani Jain, Utkarshni, Manjit Kaur Rana

Introduction: Myeloid sarcoma is also known as chloroma or granulocytic sarcoma or extramedullary myeloid cell tumor. These are rare tumors characterized by the occurrence of extramedullary tumoral masses exhibiting proliferation of myeloid precursor cells. Its estimated incidence is 0.7/million in children and 2/million in adults. It has a slight male predominance with M:F ratio- 1.2:1. The age of patients presenting with myeloid sarcoma is highly variable, ranging from 1 to 81 years old. It can involve any site of body, more common of which are skin, bone, soft tissue, gastrointestinal tract, respiratory tract, lymph nodes etc. It can occur de novo or can be associated with AML or MPNs. The size of the lesion can vary from 2 to 20 cm.
Case description: A 41-year-old male who presented with soft tissue swelling on the back measuring 10 × 10 cm from 1.5 months. On clinical examination, swelling was diffuse, soft, non-tender simulating lipoma. PT/INR studies were normal. However, no CBC report was available. FNA was performed from the back swelling and smears were highly cellular and showed cells of myeloid series including myeloblasts, myelocytes, meta—myelocytes and mature neutrophils. Few lymphocytes and nucleated red blood cells were also present in the smear. Afterwards, CBC along with PBF was performed which revealed TLC- 4,28,000/ul, Hb- 6.9 gm/dl, Platelets-2,13,000/ul. Peripheral blood smear examination also showed increase in cells of myeloid series. Bone marrow aspiration and biopsy was also performed which showed the same findings as above.
Conclusions: Myeloid sarcoma can be a presenting feature of underlying haematological malignancy, CML in this case. The cytomorphological findings along with flowcytometry can help in detection of the same.

Thrombosis

Thromboelastography Determined Fibrinolysis Shutdown And Its Correlation With Complications And Outcomes In Patients With Coronavirus Disease 2019

Tushar Sehgal, Mukul Aggarwal, Upendra Baitha, Gaurav Gupta, Bindu Prakash, Ganesh Kumar V, Ashutosh Biswas, Shalimar

Introduction: Coronavirus disease 2019 (COVID-19) causes abnormalities in the hemostatic system, which are referred to as COVID-associated coagulopathy. Whole blood viscoelastic studies, such as thromboelastography (TEG), are the best way to determine the dynamics of clot formation. The hypercoagulable state seen in COVID-19 patients is aggravated by a severe state of "fibrinolysis shutdown," which is caused by overexpression of plasminogen activator inhibitor 1 and thrombin activated fibrinolysis inhibitor.
Aims & Objectives: We aimed to study the coagulation pattern in COVID-19 patients on TEG and explored the predictors of outcomes in our patients.
Materials & Methods: An automated thromboelastogram was used to perform TEG on 28 COVID-19 patients. Based on TEG parameters such as R-time, K-time, alpha angle, maximum amplitude (MA), and clotting index (CI), the hemostatic state was classified as hypercoagulable in 17 (63 percent), hypocoagulable in 2 (7 percent), or normal in 8 (30 percent) patients.
Result: Twenty-seven patients were included, with a median age of 50 years (IQR 40–60 years) and a male to female ratio of 0.9:1. COVID-19 was mild in 6 (22.2%), moderate in 2 (7.4%), and severe in 19 (70.4%) patients. Fibrinolytic shutdown was detected in 4 (15%) patients. TEG analysis revealed that they were hypercoagulable. All four patients showed high D-dimer levels and a LY-30 of 0%, as well as decreased K-time and increased alpha angle or MA.
Conclusions: Despite thromboprophylaxis, hypercoagulable states and severe inflammatory states are prevalent in COVID-19 patients. Patients with hypocoagulable and hypercoagulable conditions had greater 28-day mortality than those with normal coagulation. (Log-rank test, P = 0.002). Only two of the four patients who had their fibrinolytic systems turned down survived. These patients had no clinically evident thrombosis.

D Dimer Levels In Children With Kawasaki Disease: Association With Disease Activity, Thrombosis And Coronary Artery Complications

Jasmina Ahluwalia, Sangamitra Rajasekaran, Ankur Kumar Jindal, Varun Uppal, Narender Kumar, Surjit Singh

Introduction: Kawasaki disease (KD) is the commonest childhood vasculitis. Coronary artery aneurysms (CAA) develop in 20–25% of untreated children of KD. Early diagnosis and treatment with IVIg significantly reduces the risk of development of CAA. IVIg resistance (IVIgR) seen in 10–20.
Aims & Objectives: To assess the role of D dimer levels in children with KD with regards to IVIg resistance and development of coronary artery aneurysms.
Materials & Methods: 48 children aged less than 15 years were enrolled from the Pediatric Rheumatology clinic. Their clinical details were recorded, cardiac echocardiography and lab tests were performed in accordance with the existing protocol. D dimer levels were estimated in 32 newly diagnosed KD children aged ≤ 15 years. Follow-up D dimer levels were available in 22 children. A separate group of 26 known cases of KD who visted the clinic during the period of the study were recruited and D-dimer was assessed once anytime during their follow up visits. The D Dimer assay was was performed using the HemosIL D dimer HS kit on The ACL TOP

coagulation analyser. Patients not responding to IVIg were included in the group of IvIg resistance. The D dimer levels were compared between active and quiescent cases, those with and without cardiac complications and IVIg resistance.
Result: D dimer levels were significantly higher with disease activity (median- 536.5 ng/ml) vs. in inactive phase (during follow up, median- 107.5 ng/ml), $p < 0.001$. D dimer level of 190.5 ng/ml could predict activity with a sensitivity of 81% and specificity of 83%. Though D dimer levels were higher in active KD patients with IVIg resistance (median- 860 ng/ml) vs IVIg responsive patients (median- 531 ng/ml) and in those with CAA (median- 1227.5 ng/ml) vs those without (median- 457 ng/ml), statistical significance was not obtained (p values 0.583 and 0.134 respectively).
Conclusions: D dimer is a reliable predictor of disease activity in KD. Though D dimer levels at admission were increased in patients with CAA and IVIgR, their clinical utility in predicting the same is uncertain and needs validation in a larger cohort.

Real Time PCR Based Detection Of The Venous Thromboembolism Associated Genetic Variations: Factor V Leiden And MTHFR C677T Polymorphism

Ravi Ranjan, Kamal Kishor, Amit Sharma, Hareram Pandey, Jasmita Dass, Mukul Aggarwal, Seema Tyagi, Manoranjan Mahapatra

Introduction: Factor V Leiden mutation (F5 c.1691G > A; p.R506Q; rs6025) and the MTHFR C677T (rs1801133) polymorphism are the major inherited risk factors of venous thromboembolism manifested as deep venous thrombosis or pulmonary embolism and have been further associated with a higher risk of myocardial infarction, stroke, and miscarriage. Traditionally, the detection of known point mutation is done by PCR–RFLP which is often time consuming and cost-intensive.
Aims & Objectives: We aimed at establishing a real-time PCR protocol for the detection of the venous thromboembolism associated genetic variation i.e. factor V Leiden (F5 c.1691G > A; p.R506Q) and MTHFR C677T polymorphism.
Materials & Methods: The conventional method has four steps including PCR followed by gel electrophoresis and further restriction endonuclease digestion and finally gel electrophoresis again to analyse the results whereas the Real Time PCR method utilizes the single step TaqMan primer probe assay (snp Biotechnology, Ankara Turkey). Factor V Leiden mutation and MTHFR C677T polymorphism were determined in 225 citrate samples from venous thromboembolism patients using both methods.
Result: Validity score values of genotype calls using RT-PCR were similar and did not significantly differ compared to those using PCR–RFLP. Genetic variant analysis of 225 samples showed a negligible PCR dropout rate (one in 450 reactions) and were in 100% concordance with results obtained by conventional genotyping. The reported method is reliable, cost-effective, and accelerates time to result supporting rapid clarification of a potential underlying genetic background of venous thromboembolism.
Conclusions: We successfully established a robust and valid real-time PCR protocol for the detection of the venous thromboembolism associated gene variations F5 c.1691G > A (p.R506Q) and MTHFR C677T polymorphism. The described method is easily reproducible in every laboratory with real-time PCR technology and may therefore stimulate further research and assist the clinician in the diagnosis of inherited venous thrombosis.

A Tricky Case Of Recurrent Venous Thromboembolism- May-Thurner Syndrome

Harika Padamata, Harshit Khurana, Nazneen Ps, Dhanalaxmi, Pranati Swain, Uday Yanamandra

Introduction: May Thurner syndrome is a rare condition in which patient develops iliofemoral deep venous thrombosis due to an anatomical variant in which right common iliac artery overlies and compresses left common iliac vein against lumbar spine. Seen in 2–5% of all patients presenting with Deep venous thrombosis. Actual prevalence- 14 to 32% (as per autopsy studies).
Aims & Objectives: In this article, we will discuss a case of Recurrent venous thromboembolism.
Materials & Methods: 33-year-old male, with history of multiple episodes of Deep venous thrombosis involving left lower limb who was on therapeutic dose of DOAC had presented with 05-day history of acute onset breathlessness, hemoptysis and pleuritic chest pain. He had tachycardia, tachypnea, hypertension, and absent breath sounds in left infra-axillary area. His D-Dimer levels were high (1540 ng/ml). His CD55, CD59, tumor markers, ANA, C3, C4, c-ANCA, p-ANCA were negative. JAK2V617F & EXON12 mutation were not detected. Bone marrow study, procoagulant work up revealed no abnormality. His ECG showed sinus tachycardia & LVH. His 2D-Echo was normal. Contrast enhanced pulmonary angiography suggestive of acute pulmonary thromboembolism of segmental & subsegmental pulmonary artery branches of left lower lobe & left upper lobe. Contrast venography revealed left lower limb chronic deep venous thrombosis with thrombosis up to left iliac vein- May-Thurner-Syndrome. Our final diagnosis- Recurrent Pulmonary thromboembolism, May-Thurner-Syndrome. He was managed with left iliac vein stenting with successful recanalization and oral anticoagulants and haven't had recurrence of Venous thromboembolism ever since.
Result: Arterial venous anatomical variants like May Thurner needs consideration and evaluation for, in the event of recurrent Venous thromboembolism. Never considered up front, due diligence and high index of suspicion can prevent life threatening venous thromboembolic events.
Conclusions: This case highlights the cause of recurrent thromboembolism due to anatomical variation in the vasculature.

Mitochondrial DNA Variants And Venous Thromboembolism

Iti Garg, Swati Srivastava, Nilanjana Ghosh, Babita Kumari, Rajeev Varshney

Introduction: Emerging multi-dimensional role of mitochondria in various diseases showed that mitochondrion is connecting bridges between diseases causing factors and human pathophysiology. As a central player in the regulation of cellular metabolism and a powerful controller of cellular fate, mitochondria functionality has been explored to some extent. Mitochondrial dysfunction as a factor playing a central role in the pathogenesis of several chronic human diseases like cardiovascular disease (CVD) has been studied.
Aims & Objectives: Mitochondria's integral role in platelet activation and initiation in the thrombus formation has been well documented. Under present study, whole exome sequencing was performed to determine association of mitochondrial variants with venous thromboembolism (VTE).
Materials & Methods: Present study was performed on Indian Army personnel as per ethical guidelines and written consent was obtained from them at the time of study. Cohort of Thrombosis patients along with sex and aged matched healthy control subjects was taken. Basic demographic and physiological information were collected. Peripheral blood was collected in EDTA vacutainers and DNA was isolated from all samples. Isolated DNA was quantified as well as quality of

DNA was also checked. Total forty samples which were consisted of high altitude VTE (n = 6), Sea level VTE (n = 15) and healthy control individuals (n = 19) were undergone exome sequencing. Bioinformatic analysis was done followed by statistical analysis.
Result: A total of 180 mitochondrial variants with distinct rsID were found in whole exome sequencing. Further analysis of these variants were done based on the OR > 1 in diseased state compared to high altitude VTE and sea level VTE. Sixteen variants showed higher OR value in the diseased state compared to the high altitude and sea level patients. Bioinformatics analysis showed these mtDNA variants of linked with MT-ND1, MT-ND3, MT-ND4, MT-ND5, MT-TL2, MT-CO1 and MT-CO2 genes which play pivotal role in mitochondria machinery.
Conclusions: Study comprehends that presence of theses mitochondrial DNA (mtDNA) mutations in patients lead to mitochondrial dysfunction which may be associated with the pathophysiology of the venous thromboembolism.

Identification Of Genetic Risk Variants Associated With Susceptibilty To Venous Thrombosis In Indian Population: A Case Control Study

Swati Srivastava, Babita Kumari, Iti Garg, Rajeev Varshney

Introduction: Venous thrombo-embolism (VTE) is a multifactorial disease having two main clinical manifestations, deep vein thrombosis (DVT) and pulmonary embolism (PE), the later one being potentially fatal. VTE occurs due to abnormal alterations in blood flow, involving either acquired or genetic factors. Although it is acertain that genetic risk factors contribute to susceptibility towards VTE, however the precise role of variants in candidate genes remains obscure.
Aims & Objectives: Present study aims to identify genetic variants for VTE susceptibility.
Materials & Methods: We analyzed 35 mutations in 22 candidate genes, previously linked to VTE, including pro-coagulants, coagulation factors and anti-coagulants. Study participants included Indian Army personnel and were divided into two groups, (i) those with confirmed VTE diagnosis (patients, n = $\sim$ 150) and (ii) healthy controls (n = $\sim$ 230). Briefly, peripheral blood was collected in EDTA tubes and DNA was extracted, followed by qualitative and quantitative check. Desired gene sequences were amplified using specific primers followed by digestion with restriction enzymes (PCR–RFLP). Genotypic and allelic frequencies for each gene were determined by comparison of digested bands on agarose gel. Graphpad prism was used to perform statistical analysis.
Result: Variants which showed statistically significant change ($p < 0.05$, $OR < 2.0$) in frequency of occurrence between VTE patients and controls included rs3736456 in CYP450, rs1799853 and rs1057910 in CYP2C9, T1325C and C807T in GP6 and rs3742262 and rs1926447 in TAFI gene. Other variants that showed significant change at genotypic level include 3 SNPs of VKORC1 gene rs9923231, rs9934438 and rs2884737; Type11 mutation G/A in F11 gene, rs1801133 in MTHFR, 844 G/A in PAI-1, 455 G/A in β-fibrinogen, rs2071942 and rs1800541 in EDN-1 gene and C/T in SERPINC1 gene. Nine other variants were found to be monomorphic in our study.
Conclusions: Present study identified specific variants in lipid metabolism gene (CYP450), gene involved in warfarin metabolism (CYP2C9 and VKORC1), gene involved in platelet aggregation (GP6), thrombin-activatable fibrinolysis inhibitor (TAFI), coagulation factor 11, anticoagulant gene (SERPINC1), vasoconstrictor (EDN-1), gene involved in homocysteine metabolism (MTHFR) and blood clot formation (β-fibrinogen). These variants might play a significant role in VTE susceptibility and may be studied to evaluate individual's risk towards VTE.

Is Undected Inherited Thrombophilia The Hades Of COVID-19?

Divya Khatana, Poonam Rani

Introduction: Thrombophilia is a co-morbidity in the general population with a prevalence of 5%. However, it is not known whether it affects severity of COVID-19. It may be possible that undiagnosed thrombophilia exaggerates an already prothrombotic state in COVID-19 patients and hence, results in severe disease.
Aims & Objectives: To study the association of underlying thrombophilia with severity of COVID-19 in post COVID patients after a minimum of 6 weeks of recovery.
Materials & Methods: Eighty RT-PCR confirmed adult covid patients (40 severe,40 non-severe) post 6 weeks of recovery were included. The venous blood in EDTA and citrate vials was tested for complete blood counts and coagulation parameters such as Prothrombin time, activated partial prothrombin time, Lupus anticoagulant by dRVVT, Antithrombin-III, Protein C and S).
Result: 6/40 patients had Protein C deficiency in severe category and none in non severe. 7/40 patients had Protein S deficiency in severe category and 1 patient was deficiant in non-severe group.
Conclusions: Thrombophilia was detected significantly in patients with severe COVID even after 6 weeks of recovery, indicating that undetected underlying thrombophilia could be a factor affecting disease severity. The common abnormalities detected were Protein C & S deficiency. This is a novel finding which needs to be explored further with extensive thrombophilia profiles. The influence of underlying thrombophilia in patients who succumbed to the disease is not known and cannot be known retrospectively. However, the extrapolation of this study suggests that thrombophilia may be an important influence on severity and mortality.

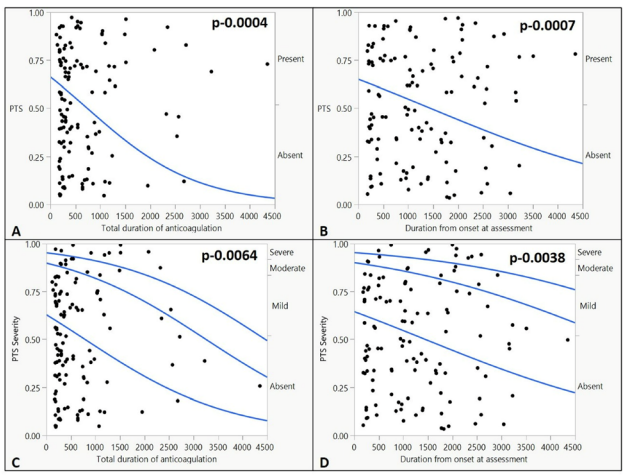
Table Inherited thrombophilia screen in severe and non-severe COVID patients post recovery. (N-80)

Lab Parameters	Severity	Mean Values	Std. Deviation	P value
PROTEIN C (%)	Severe	79.55	34.11	0.002*
	Non Severe	99.40	17.99	
PROTEIN S (%)	Severe	98.82	27.87	0.007*
	Non Severe	114.02	20.73	
AT-III	Severe	82.42	19.03	0.096
	Non Severe	89.40	17.99	

Prevalence and Clinical Characteristics Of Post-Thrombotic Syndrome in Young Male Patients with Dvt: An Experience From Real-World Settings

Sushma Yendamuri, Uday Yanamandra, Rajen Kapoor, Suman Pramanik,Kundan Mishra, Revanth Boddu, Rajat Bahl, Ankur Ahuja, Harshit Khurana

Introduction: Post-thrombotic syndrome (PTS) is a chronic debilitating complication, occurring in around 20–50% of patients with deep vein thrombosis (DVT). The prevalence, risk factors for the presence or severity of PTS, and Villalta components from real-world settings are not well studied.
Aims & Objectives: We aimed at studying the epidemiology and clinical characteristics of PTS in young men with DVT in real world settings. The objectives of the study were to assess the distribution of various components of VILLALTA scale, the incidence and severity of PTS, and their association with several identified risk factors.
Materials & Methods: This is a cross-sectional, single-center, observational study (n-123) conducted over two years. Patients were assessed for the development of PTS using the Villalta scale. Also, the presence/severity of PTS and components of the Villalta were related to various risk factors (age, BMI, total duration of anticoagulation, the extent of DVT, high altitude, antiplatelet prophylaxis, or duration from onset to assessment). JMP 15.0 was used for statistical analysis.
Result: The median age of the study population was 36 years, with 47.97.
Conclusions: PTS develops in up to half of the patients with DVT, with none of the studied risk factors showing any significant relation to the presence and severity of the PTS.



Study of Immature Platelet Fraction, Mean Platelet Volume And Interleukin-6 in Acute Coronary Syndrome

Tejus Behl, Vijay Kumar

Introduction: Acute Coronary Syndrome (ACS) includes ST-Elevation Myocardial Infarction (STEMI), non-ST Elevation Myocardial Infarction (NSTEMI), and Unstable Angina (UA). Platelet indices are simple and reliable, and are emerging as biomarkers of cardiovascular events that might potentially help in stratification of risk. Inflammation also seems to play an important role in ischaemia/reperfusion injury that follows STEMI.
Aims & Objectives: The present study was undertaken to estimate the levels of immature platelet fraction (IPF), mean platelet volume (MPV) and interleukin-6 (IL-6) in ACS and to find out the association and correlation of these parameters with ACS.
Materials & Methods: This was a comparative study conducted in the Department of Medicine, Department of Pathology and Department of Biochemistry, ABVIMS & Dr RML Hospital, New Delhi. 70 patients of ACS fitting the Universal criteria of Myocardial infarction were included and were sub-divided into Unstable Angina (UA), Non ST-Elevation Myocardial Infarction (NSTEMI).
and STEMI. 70 controls were also included in the study. MPV and IPF were determined on hematology analyser. IL-6 was measured using ELISA method.
Result: In all the subgroups of ACS; MPV, IPF and IL-6 were significantly increased as compared to controls. For predicting STEMI, ROC curve showed excellent discriminatory power of MPV(fl) (AUC 0.855; 95% CI: 0.774 to 0.915). Significant positive correlation was seen between IPF and MPV in all the three subtypes i.e. STEMI, NSTEMI and UA with correlation coefficient of 0.297, 0.531 and 1 respectively.
Conclusions: The results of the present study appear to substantiate that MPV, IPF and IL-6 are significant ancillary biomarkers in the evaluation of ACS. Patients with acute coronary syndromes had higher MPV, IPF and IL-6 when compared to controls. Therefore measurement of platelet volume indices and inflammatory biomarkers may be of help in differentiating the individuals with cardiac and non-cardiac chest pain and hence could benefit from early interventions.

Study Of Plasma D-Dimer Level and Mean Platelet Volume in Patients with Chronic Urticaria and Their Correlation with Disease Severity

Kaushal Kumar, Sadhna Marwah

Introduction: Urticaria is a common skin disease impacting negatively on multiple aspects of patients' lives. It is characterized by the development of wheals, angioedema, or both. It can either be acute or chronic. Causes of chronic urticaria remain obscure. Laboratory parameters or biomarkers can be a predictor of severity of Urticaria.
Aims & Objectives: To Study role of plasma D-dimer level and mean platelet volume in patients of chronic urticaria and their correlation with disease severity.
Materials & Methods: An observational cross-sectional study on 32 patients of chronic urticaria seen in allergy-clinic, during past 1-year span and 32 age and sex-matched control was carried out. Demographic details, duration of the disease, urticaria activity score (UAS), plasma D-dimer levels, mean platelet volume were included.
Result: It was observed that 71.9% of the patients had UAS score of 29–42 which indicates that presence of severe activity urticaria. However, mild and moderate activity urticaria was present in only 12.5% and 15.6% of the patients with a score of 7–14 and 15–28 respectively. It was observed that, 59.4% of the patients had D-dimer levels of < 250 ng/ml while 40.6% of the patients had > 250 ng/ml. However,100% of the patients under the controls had D-dimer level of < 250 ng/ml. It was observed that 75.
Conclusions: There was statistically significant difference in D-dimer level (p value < 0.001) and mean D-dimer level (p value 0.021) when compared between the two groups (cases and controls. MPV (p value < 0.001) and mean MPV (p value < 0.001) was higher in urticaria patients as compared to controls and was statistically significant. Correlation was done between UAS with MPV and UAS with D-dimer for cases by Pearson correlation. It was observed that there was no significant correlation found between UAS with MPV ($r = 0.245$, $p = 0.176$) and D-dimer ($r = 0.227$, $p = 0.221$). Evaluation of D-dimer levels and MPV can be useful to predict the disease, severity and to manage patients appropriately.

Correlation of Hemostatic Abnormalities With Clinical Risk Score for Thromboembolic State in Patients With Carcinoma Lung: A Cross Sectional Study

Jadda Sai Charan Teja, Uday Yanamandra, Tvsvgk Tilak, Medapati Sudeep Veer

Introduction: Lung cancer patients are prone to venous thromboembolism. Currently, there is lack of data regarding clinical risk scoring in patients with cancer from India.
Aims & Objectives: To study the clinical risk scoring (Khorana Score) in lung cancer patients.
Materials & Methods: This is a cross-sectional single centre descriptive study from Western India. Patients of lung carcinoma visiting oncology OPD/IPD during study period (Oct 20-Sep 22) were screened. Patients with pre-existing coagulation abnormality, other co-existing malignancies, & taking anticoagulant therapy. All included patients were subjected to platelet count, hemoglobin level, leukocyte count & anti-cardiolipin antibodies and correlated with Khorana risk scoring for thromboembolism. Data was analyzed using JMP ver 16.0.0.
Result: Study population included 58 patients with a mean age of 56.44 ± 11.49y and male preponderance (59%). Among those 57% were adenocarcinoma, 34% non-small cell carcinoma (NSC), and 9% were small cell carcinoma (SCC) with a majority having metastatic disease (64%). Ten percent patients succumbed to illness during the study period. Mean Khorana risk score was 1.43 ± 0.8 in the study population. Khorana score was not statistically different in different types of lung Ca (NSC-1.35, AdenoCa-1.51, SCC-1.0; p-0.22), metastasis status (p-0.13), survival status (p-0.5), gender (p-0.82). Components of Khorana score amongst study population were Hb-11.5 ± 1.33 g/dl, TLC-8648 ± 2248/mm^3, Platelet count-2.52 ± 0.93 lakh/mm^3 with a mean BMI of 22.42 ± 1.73 kg/m^2. None of the patients had positive anti-cardiolipin antibodies.
Conclusions: Patients of Adenocarcinoma lung have high Khorana risk score for VTE, compared to other types of Lung cancers.

Stem Cell Transplantation

Frequency Distribution of HLA-B Leader Motif Among Healthy Unrelated Individuals From India: Insights for Better Donor Selection

Selma Z DSilva, Manisha Tambe, Jyoti Rajak, Andrea S Pinto, Bansi Parsaniya, Meenakshi Singh

Introduction: HLA B is the most polymorphic HLA gene having a total of 9000 alleles as of August 2022. These variations are implicated in varied immune responses. A dimorphic variation in HLA B gene at position -21 has been associated with predicting hematopoietic stem cell transplant outcomes, and survivorship. Reports suggest that the risk of aGvHD is higher in HLA B mismatched HSCT, when the patient carries an M leader sequence and also when there is a mismatch in the patient/donor B leader.
Aims & Objectives: The aim of this study was to evaluate the HLA B leader allele and genotype frequency in unrelated healthy donors from Western India.
Materials & Methods: A total of 416 unrelated healthy individuals were typed for HLA B loci using the Next Generation sequencing technology (GenDx, Utrecth, The Netherlands). The final library with concentration of 1.1 pm was run on the Illumina MiniSeq system (Illumina, USA). HLA B leader allele and genotype was assigned using the freely available software B leader tool (https://www.ebi.ac.uk/ipd/imgt/hla/matching/b_leader/) with HLA B typing of the indi vidual as the input data. Allele Frequency and Genotype frequency was estimated using the frequency functions in Microsoft excel.

Table 1 Allele and Genotype Frequency of HLA B leader peptide in Healthy unrelated individuals from India

Allele	Frequency
T	88.3%
M	11.7%
Genotype	**Frequency**
TT	78%
MT	20%
MM	2%

Result: From the 832 HLA B alleles in the study, a total of 55 unique alleles were identified. The most common HLA B allele in this cohort was found to be HLA B*40:06:01, which carries the T group. It was observed that majority of the individuals carried the T (Threonine) allele (88.3%) as compared to the M (Methionine) allele (11.7%). Similarly, the TT haplotype was the most frequent (78%), followed by the heterozygous MT (20%) and the homozygous MM haplotype (2%). [Table 1].
Conclusions: This data provides an insight to the availability of HLA B permissive and non permissive donors in the Indian population. An understanding of the B leader allele in the donor will help in identifying the best donor for HSCT with reduced risk of aGvHD.

Evaluation of Structural Changes in Novel HLA Alleles Arising from Point Mutations

Andrea S Pinto, Selma Z DSilva, Manisha Tambe, Jyoti Rajak, Bansi Parsaniya, Meenakshi Singh

Introduction: The Major Histocompatibility Complex (MHC) which includes the classical class I (HLA-A, B and C) and class II (HLA-DRB1, DQB1 and DPB1) genes are polymorphic in nature. These polymorphisms lead to the discovery of novel HLA alleles. Novel alleles are generated by molecular mechanisms such as point mutations, crossovers, intralocus and interlocus recombination. In class I, exon 2, 3 and 4 encode the extracellular domains α1, α2 and α3 respectively whereas exon 5 encodes the transmembrane region. In class II, the extracellular domains are encoded by exon 2 and 3. Amino acid mutations in these regions could lead to a change in the protein conformation and hence affect the peptide binding properties.
Aims & Objectives: To evaluate the change in conformation in the novel HLA alleles.
Materials & Methods: A total of 1021 healthy donors were HLA typed for both Class I and Class II alleles from February 2021 to August 2022 using Next generation sequencing (NGS) technology (GenDx, Utrecht, Netherlands).
Result: A total of 17 novel alleles were discovered in the 1021 donors who were HLA typed, which could have likely emerged from point mutations in the exonic region. Of these, 11 were in class I and 6 in class II genes. In class I, 7 novel alleles having point mutations caused non-synonymous amino acid changes whereas the remaining 4 novel alleles are a result of synonymous amino acid changes. All the novel alleles of class I discovered in exon 2 and 5 belong to HLA-A loci, exon 3 belong to HLA-B and exon 5 belong to HLA-B and HLA-C loci.
In class II, 3 novel alleles having point mutations caused non-synonymous amino acid changes, 2 novel alleles caused synonymous amino acid changes and one is a result of a nucleotide deletion which

causes a shift in the reading frame. Most of the exonic mutations were seen in HLA-DRB1 which is one of the most polymorphic Class II gene.

Conclusions: This study highlights the need for NGS HLA typing to identify such novel alleles which can result in structural and functional changes thus affecting the protein expression.

MHC Class	Loci	Nearest match	Exon	Nucleotide change	Amino acid change	Change in amino acid polarity	Officially assigned allele
Class I	A	24:02:01:01	2	294C > G	T30R	Uncharged, polar to positively charged, polar	*HLA-A*24:537*
	A	33:03:01	2	406G > A	K68K	No change	IMGT certification awaited
	A	68:01:02:02	2	351C > T	P50L	Neutral non-polar to hydrophobic non-polar	*HLA-A*68:175:01* (Confirmatory sequence)
	A	68:175:01	2	232C > T	T10T	No change	*HLA-A*68:175:02*
	B	07:02:01:01	3	969G > A	G174E	Neutral, non-polar to negatively charged, polar	*HLA-B*07:461*
	A	02:01:01:01	4	1076G > A	T228T	No change	IMGT certification awaited
	B	40:06:01:02	4	C > T	P184L	Neutral non-polar to hydrophobic non-polar	*HLA-B*40:387* (Confirmatory sequence)
	C	03:04:01:02	4	1618G > T	A195S	Hydrophobic, non-polar to uncharged, polar	*HLA-C*03:599*
	C	12:02:02:01	4	1611T > C	H192H	No change	*HLA-C*12:02:47*
	A	02:09:01:01	5	2058A > G	S312G	Uncharged, polar to neutral, non-polar	*HLA-A*02:1013*
	A	68:01:02:02	5	2014T > G	G297V	Neutral, non-polar to aliphatic, non-polar	*HLA-A*68:287*
Class II	DRB1	14:04:03	2	5589C > A	T77N	Uncharged, polar to	*HLA-DRB1*14:50:02*
	DRB1	14:152N	2	G > Del	G73-	Deletion	IMGT certification awaited
	DRB1	14:54:01:01	3	7898T > G	S104A	Uncharged, polar to hydrophobic, non-polar	*HLA-DRB1*14:245*
	DRB1	10:01:01:01	3	8104C > A	T172T	No change	IMGT certification awaited
	DPB1	02:01:02:03	3	9011C > T	I146I	No change	*HLA-DPB1*02:01:61*
	DPB1	02:01:02:05	4	9755G > A	G212E	Neutral, non-polar to negatively charged, polar	IMGT certification awaited

Lymphodepletion Pattern Among Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplantation Receiving A Fludarabine Based Conditioning Regimen

Nayanthara K B, Aswin Anand Pai, StallonIllangeswaran, Uday P Kulkarni, Vikram Mathews, Poonkuzhali B

Introduction: Allogeneic Hematopoietic stem cell transplantation (HCT) is the only potential curative therapy for most haematological disorders. Fludarabine (Flu) based conditioning regimen have improved HCT outcomes in both malignancies and non-malignant conditions. We did not find any association between plasma fludarabine exposure on HCT outcomes in patients receiving Flu based regimen (Mohanan et al. 2017, 2015, 2016). Fludarabine acts as the immune suppressive component of the conditioning regimen. Hence, we hypothesized that the percentage reduction in T-lymphocytes before vs. end of conditioning would serve as a pharmacodynamic marker rather than plasma fludarabine exposure itself.

Aims & Objectives: To evaluate the extent of lymphodepletion in different fludarabine based conditioning regimen in patients undergoing HCT.

Materials & Methods: Patients who underwent allogenic HCT with Flu in combination with different alkylating agents: Busulfan (Flu/Bu, n = 32), Cyclophosphamide (Flu/Cy, n = 18), Melphalan (Flu/Mel, n = 18) and Treosulfan (Flu/Treo, n = 14) as conditioning regimen between 2020 and 2022 were included in the study. Peripheral blood samples from the patients were collected before flu administration and at the end of the conditioning. The expression of lymphoid surface markers CD3 and CD56 was assessed using flow cytometry and the extent of lymphodepletion (CD3 + T cells, CD56 + NK cells and CD3 + CD56 + NKT cells before vs. end of conditioning) was compared across the different groups. Also, the % lymphodepletion was compared with engraftment, chimerism/graft rejection status among patients receiving different flu-based regimen.

Result: Comparable reduction of immune cells was observed at end of conditioning in all four different combinations. Maximum reduction of CD3 + T cells was observed in Flu/Treo cohort (median percentage reduction of 95.8%; range: 24.4%–99.4%) and lowest in patients with Flu/Cy regimen (median 77.15%; range: 15.1%–97%). CD3 + CD56 + NKT cells and CD56 + NK cells did not show significant reduction when compared across all the 4 cohorts. There was no association between the extent of lymphodepletion and engraftment, chimerism or graft rejection.

Conclusions: Extent of lymphodepletion in patients receiving flu-based regimen was not associated with HCT outcome, similar to fludarabine systemic exposure. Further analysis combining fludarabine exposure along with lymphodepletion post conditioning on HCT outcome is ongoing.

PNH presentation as Anaemia of Nutritional Deficiency

Manoj Saini

Introduction: Paroxysmal Nocturnal Haemoglobinuria(PNH) is a rare acquired clonal hematopoietic stem cell disorder from mutations in the phosphatidylinositol glycan class A gene (PIGA) located on X chromosome whose presentation is a balance between three components: intravascular haemolysis, bone marrow failure and thrombotic complications (1–4).

It affects male and female in equal numbers. The Prevalence is estimated to be between 0.5 and 1.5 per million people in the general population. The median age at diagnosis is 30 years.

PIGA mutation leads to deficiency of 2 GPI anchored proteins CD55 & CD59 that leads to complement mediated haemolysis and other PNH manifestations. PNH is a chronic disease. Eculizumab and improved supportive care have resulted in prolonged survival to more than 75%. (2).

Nutritional deficiency, especially iron deficiency, and hemoglobinopathy is quite common in eastern part of India. Many a times it is difficult to suspect and evaluate for PNH in areas where these diseases are common.

Case Report: We report a case of 48 years old male who presented to us with complain of easy fatigability and yellowish discoloration of eyes for 4 months. He did not have any co-morbidities. He did not give any history of cardiorespiratory illness or bleeding in any form or pain abdomen or dysphagia or fever or recent immunization. There was no previous history of transfusion. Patient used to take a mixed Indian diet and had a normal bowel and bladder habit.

Patient was evaluated with all routine investigations which showedHb- 6.3 g/dl, MCV- 80 fl, MCH- 27 pg,TLC- 334o/cmm with normal differentials, TPC- 1.3lakhs/cmm, PS- anisopoikilicytosis with polychromasia without any abnormal cells. Liver function test showed indirect hyperbilirubinemia without transaminitis. DCT &ICT were negative. Reticulocyte counts were raised(8.6%). Serum vitamin B-12 levels were initially (Feb,2022)192 pg/ml,then patient was given injectable Vit b12 and repeated level (on June,2022) was 512 pg/ml. LDH level was 3100 U/L. S Ferritin-12 ng/ml, S Iron-35mcg/l TIBC-180.As a part of pancytopenia evaluation bone marrow examination was done which showed normocellular marrow with erythroid hyperplasia and adequate megakaryocyte without dyspoiesis.Cytogenetics and stress cytogenetics were normal. Patient was further investigated with flow cytometry for PNH which revealed PNH Type 3 RBC (total loss of CD 59); clone size was 34%. Type 2 RBC (Partial loss of CD59) 2.28% and 94% monocyte and 87.5% granulocytes were deficient in CD157. This is a patient of PNH whose presentation was as hemolysis and also pancytopenia. We transfused one unit of leucoreduced irradiated PRBC. Patient was started on oral Prednisolone along with oral iron, B12, Folate supplementation. All the blood parameters were stabilized,without further transfusion, after

two weeks of treatment. Jaundice improvedwithout any treatment. Patient was planned to start on eculizumab/Ravlizumabbut could not be started due to unavailability.

Conclusion: Anaemia due to nutritional deficiency is prevalent in most of the part of India. One should have high index of suspicion to evaluate for any associated condition for anaemia, as we saw association of PNH in a patient who presented with nutritional anaemia. Early detection and proper treatment can prevent development of more serious complications.

Hematopoetic Stem Cell Transplantation in Bihar: Our Experience

Santosh Kumar, Jitendra Kumar, Divya Krishna, Ravi Singh, Israrul Haque, Khursheed Mallick, Shivali Ahlawat, Tejindar Singh, Rabindra Nath Tagore, Shantanu Kumar, Avinash Kumar Singh

Introduction: Hematopoietic stem cell transplant both autologous as well as allogenic has been performed successfully to treat patients with different types of haematological malignancies, genetic disorders and hereditary immune deficiencies. Since 1983, Stem cell transplantation has been carried out in different institutes of India, But till 2017, no transplantation was performed in state of Bihar.
People from Bihar use to travel 1000 of kms away from their home for Treatments of haematological disorder including HCT.

HCT facility in their own has enormous impact on the accessibility, quality of care, family and social support.

Aims & Objectives: We wish to share our results and experience of Hematopoietic stem cell transplantations done in last 5 years.

Materials & Methods: Retrospective analysis of HSCT in BIHAR India From Jan 2017 to July 2022.
The dates of Transplant, clinical details data were collected from hospital medical records and analysed using SPSS software.

37 patients received myeloablative conditioning regime whereas 5 patients received immunosuppressive and less myeloablative protocol.

Sources of stem cells in case of allogenic transplant are peripheral blood blood stem cells of match siblings and in case of autologous transplant, these are peripheral blood stem cells.

Result: we have performed of 42 HCT, Commonest indications were multiple myeloma and aplastic anemia. we have done autologous20 and 22 allogenic transplant.
INDICATIONS for autologous HCT were Multiple myeoloma, NHL and indications of allogenic transplant were Aplastic anemia, AML in CR 1 and CR 2, ALL in CR 2, High risk MDS, Thalassemia major class 3, CNL in advance phase.

Other details and transplant outcome, we will share during conference.

Major challenge other than disease related, we faced were support staff training, family counselling and financial limitations. we thank our state govt. For helping our HCT patients through CMRF.

Conclusions: HCT at new center always a challange for any transplant team, but co-ordination among team traind team members, counselling of family, proper selections of pts and doner, HCT facilty cand be intitaed and can continued and can be successfully continued.

Safety and Efficacy of Granulocyte Transfusions in Life Threatening Infections in Severe Neutropenia

Sreedhar Jayakrishnan Cherulil, Kesavan MR, Sudeep V

Introduction: Infectious episodes in patients with profound neutropenia remain one of the most challenging aspects of care in patients receiving HSCT(Hematopoietic Stem Cell Transplantation) or high dose chemotherapy. Despite advances in the availability of antimicrobial agents and modern supportive therapy 13 to 60% of patients undergoing HSCT develop blood stream infections with a mortality rate that can reach up to 42% in these patients. The emergence of multi drug resistant organisms has further negated the ability of antimicrobials to combat these infections. Hence there needs to be additional modalities available in our treatment armamentarium to tackle the same. Functioning white blood cells are vital to the clearance of infections in humans, and hence the provision of granulocytes from healthy donors might lead to the early clearance of the index infection, promoting survival in these patients.
The present study aims to evaluate the safety and efficacy of granulocyte transfusions in patients with severe neutropenia and life-threatening blood stream infections.

Aims & Objectives: To study the safety and efficacy of granulocyte transfusions in patients with profound neutropenia in combating severe blood stream infections.

Materials & Methods: The present study was a retrospective observational study of 12 patients who received granulocyte transfusions in a tertiary care centre. All patients had profound neutropenia defined as $< 0.5 \times 109$/L, and a life-threatening blood stream infection defined by impending shock, hemodynamic instability or continuous fever spikes not responding to antimicrobial agents.
The 12 patients received a total of 18 GTX transfusions.

Result: The median age of the cohort was 18.75 years. The underlying diagnosis in the patients were AML in 4 patients, Relapsed ALL in 4 patients, JMML, MDS, Aplastic Anaemia and Multiple Myeloma accounted for 1 patient each. 41.6% of the cohort went for HSCT and 58.3% were receiving high dose chemotherapy. The median days of neutropenia before giving granulocyte transfusions was 15 days, The organisms isolated were Klebsiella Pneumoniae in 50% of patients 16.6% (2 patients) had Acinetobacter species isolated in their cultures while 2 patients had documented evidence of fungal sepsis with Candida species and Trichophoron Ashahii and one patient had Stenotrophomonas maltophilia isolated in blood cultures. Two of our patients were given granulocyte infusions based on clinical parameters suggestive of septic shock and did not have a causative organism isolated in cultures. The median time to resolution of infection was 5.5 days. 66.6% of the patients were free of any signs of infection at 30 days of follow up.

Conclusions: Granulocyte transfusions as an adjunct to antimicrobials in the treatment of severe neutropenic sepsis in patients undergoing HSCT or high dose chemotherapy is effective and well tolerated, and can prove especially beneficial in combating multi drug resistant organisms.

Clinical Profile Of Early CMV Reactivation (Upto Day + 90) In Patients Post Haploidentical Allogenic Peripheral Stem Cell Transplantation (PBSCT): A Single Centre Study From

Nirali Chandan Solanke, Deepak Patil, Shubh Purohit, Digambar Panchal, Meghana Sanas, Gauri Naik,Shilpa Joshi, Jitendra Khedkar, Anant KUlkarni, Chandrakant Lahane, Abhijeet Giram, Rajesh Phatale, Kannan Subramanium, Shashikant Apte

Introduction: CMV reactivation is a common complication following Allogenic PBSCT, with Haploidentical Donor being an independent risk factor. We report clinical profile of early CMV reactivation (upto day + 90) in patients who underwent Haploidentical PBSCT for various indications at our Institution.

Aims & Objectives: To study clinical profile and outcomes of CMV reactivation within first 90 days post Haploidentical PBSCT.

Materials & Methods: 52 patients underwent Haploidentical PBSCT at our centre between May 2017 and May 2022, of which 25 patients were excluded; those in whom engraftment was not achieved, Primary rejection or secondary rejection within 15 days of engraftment and those who succumbed before day + 30.
Retrospective analysis of 27 patients who underwent Haploidentical Allogenic SCT with successful engraftment, between May 2016 to May 2022 was done, for various hematological indications. Quantitative CMV PCR was used for monitoring of CMV reactivation after transplantation, at weekly basis after engraftment upto + 60 days and then fortnightly and, in addition, as and when clinical suspicion.
Result: 78% were males and 22% were females. Age of recepients ranged from 6 to 55 yrs. All recepients and Donors were positive for CMV IgG and negative for IgM. All recepients were given prophylactic IV Acyclovir from Day + 1 and IV Gancyclovir/oral valgancyclovir +—Cidofovir, as therapeutic approach. CMV reactivation within 90 days was seen in 23 (85%) out of 25 recepients, of which 20 patients had features of Skin/Gut GVHD, requiring increase in immunosuppression following which CMV reactivation occurred. Asymptomatic viremia was seen in 15 patients (65%) whereas overt CMV organ involvement was seen in 12 patients (45%), most commonly as Bone marrow suppression > Enteritis > Hepatitis > Pneumonia and Cystitis.
BKV viremia was associated with CMV cystitis. CMV associated mortality was seen in 2 patients (7.4%)—CMV pneumonia Hepatitis & Colitis.
Conclusions: Asymptomatic CMV viremia is more common which can be prevented by frequent monitoring of CMV copies, before serious life threatening organ involvement can occur.

A Rare Case Of Hereditary Spherocytosis Treated With Allogenic Hsct

Juhi Mehrotra, Santanu Sen

Introduction: Hereditary spherocytosis is a congenital haemolytic anaemia characterised by defective transmembrane proteins leading to formation of spherical RBCs in peripheral blood with greater than normal osmotic fragility causing severe anemia, jaundice and splenomegaly. Severe phenotypes remain transfusion dependent for life.
Aims & Objectives: Herein we report such a case who underwent a successful Allogenic Hematopoietic Stem Cell Transplant with a Matched Unrelated Donor.
Materials & Methods: Five-year-old girl, diagnosed with HS Type 1, born to a mother with history of 3 intrauterine deaths in first trimester all due to severe fetal anemia incompatible with life. She required 3 Intrauterine blood transfusions during, the lowest being Hb of 1 gm/dL. She was NICU graduate with prematurity and severe hyperbilirubinemia requiring exchange transfusions. Flowcytometry of Red Cell showed 70% reduction in alpha spectrin and 30.
Result: White cell engraftment at day + 13, platelet engraftment at day + 22 and chimerism of 99% at day + 18 was achieved. Complications included severe MDR klebsiella pneumonia sepsis, engraftment syndrome and CMV reactivation.
Conclusions: Day + 77 post-transplant with donor blood group, she remains transfusion independent with follow up chimerisms' being 99%. We plan to continue IS for 1 year post HSCT.

Conditioning protocol – Flu / Thiotepa / Treo/ ATG/ PT-Mtx

Weight 17 Kgs, Height 102 cms BSA = 0.71

Sr.No.	Day	Date	Drug	Dose
1.	-8	16/6/2022	**Inj. Thiotepa 136 mg**	8 mg/kg/day X 1 day
2.	-7 -6 -5 -4	17/6/2022 18/6/2022 19/6/2022 20/6/2022	**Inj Fludarabine 28.4 mg/day**	40 mg/m^2/day X 4 days
3.	-7 -6 -5	17/6/2022 18/6/2022 19/6/2022	**Inj. Treosulphan 10 g/day**	14 g/m^2/day X 3 days
4.	-4	20/6/2022	**Inj Thymoglobulin 8.5 mg/day**	0.5 mg/kg/day
5.	-3	21/6/2022	**Inj Thymoglobulin 35 mg/day**	2 mg/kg/day
6.	-2	22/6/2022	**Inj Thymoglobulin 35 mg/day**	2 mg/kg/day
7.	-1	23/6/2022	**Inj Cyclosporine 50 mg IV BD** **Rest Day**	3 mg/kg/dose IV BD
8.	0	24/6/2022	Stem Cell Infusion	
9.	+1,	25/6/2022	**Inj. Methotrexate 10.7 mg** **Start Inj. GCSF 170 mcg**	15 mg/m2 10 mcg/kg
10.	+3 +6 +11	27/6/2022 30/6/2022	**Inj. Methotrexate 7.1 mg** (with Folinic acid)	10 mg/m2

Pure Red Cell Aplasia Due To Major ABO-Incompatibility Following Haematopoietic Stem Cell Transplantation Manged By Subcutaneous Bortizomib-Case Report

Santosh Kumar, Santosh Kumar, Avinash Kr. Singh, Divya Krishna, Ankit Kumar, Shantanu Kumar, Khursid Mallick

Introduction: Pure red cell aplasia (PRCA) is an uncommon complication due major ABO-incompatility following haematopoietic stem cell transplantation. It is characterised by anaemia, reticulocytopenia and absence of erythroid precursors in a morphologically normal-appearing bone marrow.
Aims & Objectives: Rare case presentation.
Materials & Methods: Actual treated case.
Result: Here we describe a case of PRCA after Allogenic stem cell transplant that responded remarkably to treatment with subcutaneous administration of the proteasome inhibitor bortezomib.
A 45 Year old male patients, case of very severe aplastic anemia, underwent allogenic SCT, during engraftment he developed severe pure red cell aplasia which CMV reactivation, which was managed with subcutaneous Bortizomib 2 mg weekly for 4 wks, after that there was drastic improvement and successfully engraftment occurred.
Conclusions: Most cases of PRCA resolve spontaneously within weeks to months. While a small subset of patients has a protracted disease course requiring continued red blood cell (RBC) transfusions, there is no approved standard of care for PRCA.

Successful Third Haploidentical Hematopoietic Stem Cell Transplantation For Severe Aplastic Anemia After Two Times Of Primary Graft Failure: A Ride To Remember

Manthan Kathrotiya, Ankit Jitani, Hemant Menghani, Vijaykumar Shirure, Shruti Bhise, Jainee Patel, Sandeep Kheni, Velu Nair

Introduction: Severe aplastic anemia (SAA) is a life-threatening condition and hematopoietic stem cell transplantation (HSCT) is the curative option. Graft failure (GF) is one of the most serious complications of HSCT. Incidence rate of GF is around 8% in Matched Sibling Donor (MSD) HSCT and it can increase up to 25% with > 1-antigen-mismatched related donors.
Aims & Objectives: Successful third Haploidentical Hematopoietic Stem Cell transplant in a case of severe aplastic anemia.
Materials & Methods: An 8 year old male child diagnosed to have SAA in June 2021. He did not benefit from Danazol, Romiplostim,

Table 1 Transplant details

	Transplant 1	Transplant 2	Transplant 3
Donor	Father	Father	Sister
HLA match	6/12	6/12	8/12
DSA pre-transplant (max MFI)	1470	1365	Negative
Conditioning regimen	rATG + FLU + BU	rATG + FLU + CTX + MEL	FLU + THIO
Graft source	PB	PB	PB
CD34 + (*10^6) cell dose	11.8	22.45	11
GVHD prophylaxis	PT-Cy/PT-Ben + CsA + MMF	PT-Cy/PT-Ben + Tac + MMF	PT-Ben + Tac + MMF
Time of neutrophil engrafment	Failure in engrafment	Failure in engrafment	10
Time of platelet engrafment	Failure in engrafment	Failure in engrafment	14
Graft Failure	Primary (Chimerism < 10%)	Primary (Chimerism < 10%)	Engrafted (Chimerism 100% donor)

rATG-rabbit Anti-thymocyte globulin, FLU-Fludarabine, BU-Busulfan, CTX-Cyclophosphamide, MEL-Melphalan, THIO-Thiotepa, PT-Cy-Post-transplant Cyclophosphamide, PT-Ben-Post-transplant Bendamustine, CsA-Cyclosporine, Tac-Tacrolimus, MMF-Mycophenolate Mofetil.

Eltrombopeg, IVIG. He was considered for allogenic HSCT but there was no MSD or Matched Unrelated Donor (MUD) available, so haploidentical HSCT was planned with father as donor. Donor Specific Antibodies (DSA) were detected (> 5000 MFI) and desensitization was done with Rituximab and IVIG. Details of 3 transplant are given in Table 1. CMV reactivation (87,485 copies/ml) after 1st HSCT which was controlled with IVIG, Ganciclovir, Foscarnet and Cidofovir. GVHD prophylaxis used was PT-Cy/PT-Bendamustine, Cyclosporine/Tacrolimus/Sirolimus and MMF in all HSCT.

Result: He engrafted neutrophils on Day + 10 and platelet on Day + 14 post third HSCT. Chimerism was 100% donor DNA. He had BK virus reactivation (> 9,200,000 copies/ml) which was treated with Cidofovir. Patient had Acute Gut Graft Versus Host Disease (GVHD) (Grade II) on Day + 25 which was steroid dependent and required addition of Ruxolitinib for steroid sparing. He had Clostridium Difficile colitis four times which was treated with oral vancomycin and metronidazole. He had drug induced Nephrogenic Diabetes Insipidus which was treated with desmopressin. Patient is now 9 months post-transplant and continues to be on low dose steroids and sirolimus and maintains full donor chimerism.

Conclusions: Though high risk of GF with positive DSA, haploidentical HSCT is an effective curative treatment for SAA pediatric patients without MSDs or MUDs. Our case shows that timely detection and management of infection and complications is the key to success.

Outcomes of Allogeneic Hematopoietic Stem Cell Transplantation for Lymphomas: A Single-Centre Experience

Priyanshi Pchauri, Sushil Selvarajan, Uday Kulkarni, Sharon Lionel, Fouzia N A, Anup Devasia,Aby Abraham, Kavitha Lakshmi, Alok Srivastava, Vikram Mathews, Biju George, Anu Korula

Introduction: Multiple relapsed/refractory lymphomas represent a group of hematolymphoid neoplasms which have remained a challenge to treat. Effectiveness of allogeneic Stem Cell Transplantation in this population of patients has been attributed to graft-versus leukemia (GVL) effect.

Aims & Objectives: This study report experience of our center on allogeneic transplant for high risk & relapsed/refractory lymphomas.

Materials & Methods: All patients with histologically confirmed diagnosis of Hodgkin or non-Hodgkin lymphoma who underwent allogeneic HSCT from 2005 to 2022 were retrospectively studied.

Result: 49 patients underwent transplant: 20 (40.8%) had Hodgkin lymphoma and of the remaining 8 were B-Cell and 12 were T-Cell lymphoproliferative disorders. The median age was 30 yrs (Range:12–58 yrs) and 31 (63.3%) were male. The majority of patients (98%) had received 3 or more lines of chemotherapy. At the time of allogeneic transplantation, 21 (42.8%) patients were in complete remission—3(6.1%) in CR1 and 18 (36.7%) in > / = CR2. Of the remaining, 18(36.7%) were in partial remission and 10(20.4%) had refractory disease at pretransplant assessment. 36(73.5%) patients received non-myeloablative conditioning and the remaining received myeloablative regimens. This was followed by stem cell transplantation of which 35 (71.3%) received matched related donor grafts, 11(22.4%) received haplo-identical donor grafts and 3 (6.1%) received matched unrelated donor grafts. The 100-day treatment-related mortality rate was 30.6%.

The 3 yr event free and overall survival was 49.0 ± 9.4% and 53.1 ± 9.6%, respectively; mean follow-up being 33 months. On univariate analysis, presence of chronic Graft Vs Host Disease (cGvHD) was found to have statistically significant impact on outcomes. In the groups of patients in CR(All CR) and those with partial response or refractory disease at time of transplant, the EFS at 3 years was 65.9% and 31.9% respectively (p = 0.07) and OS at 3 years was 70.3% and 35.8% respectively (p = 0.08). Patients who developed cGvHD post-transplant also had significantly better survival characteristics (EFS at 3 yrs = 85% ± 11.6% Vs 21.4% ± 11.0%; p < 0 >

Conclusions: Allogenic transplant has a remarkable disease control in high risk and relapse/refractory lymphoma. Factors that had favorable impact on outcomes were pre-transplant remission status and presence of chronic GvHD.

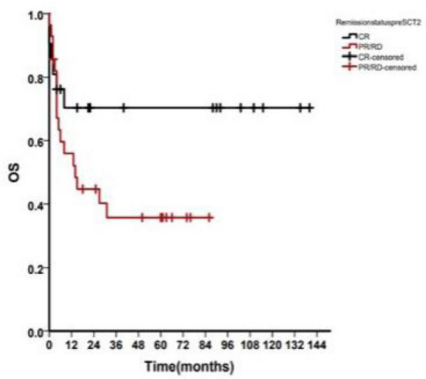


Fig 1: OS in patients in CR Versus those not in CR (PR/RD) at time of transplant. [p=0.08]

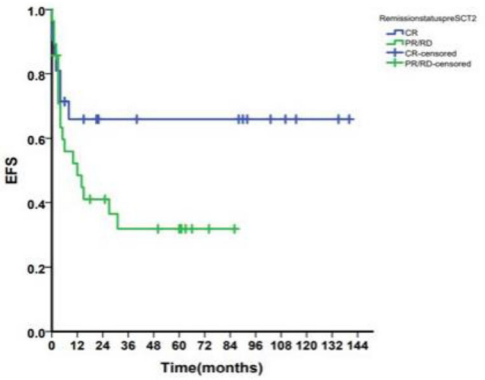


Fig 2.: EFS in patients in CR Versus those not in CR (PR/RD) at time of transplant [p=0.07]

Clinico-Hematological Profile, Flow Cytometric Analysis and Allogeneic Stem Cell Transplantation in Five Unusual Cases of Aplastic Anemia (With or Without Paraoxysmal Nocturnal Hemoglobinuria/Myelodysplastic Syndrome): Challenges

Shilpi More, Saroj Chauhan, Geetika Sharma, Nimisha Sharma,Rahul Bhargava, Sanjay Rai, Sonu Choudhary, Tathagata Chatterjee

Introduction: Aplastic anemia (AA) is a life threatening hematopoietic disorder associated with significant morbidity and mortality. There is a strong relationship between acquired AA, Paraoxysmal nocturnal hemoglobinuria (PNH) and Myelodysplastic syndrome (MDS) with concomitant diagnosis of PNH in 35–40.
Aims & Objectives: To study the clinico-hematological profile, PNH clones by flow cytometry, management with various conditioning regimens along with pre and post-transplant follow-up in five unusual patients of AA.
Materials & Methods: An observational prospective analytical study was conducted, comprising of five patients of AA. Three of them had PNH, of which one had associated MDS as well.
Result: All patients were male, with a median age of 32 years (Range:25-43 years). Four were fully matched and one was haploidentical. One patient had bidirectional mismatch. Four patients received reduced intensity conditioning regimen (RIC) with Flu/Cy/ATG while one received myeloablative therapy with Flu/Cy/ATG/TBI. Median CD34 dose was 6million/kg body weight of the recipient. All had successful engraftment. GVHD prophylaxis was done using Cyclosporine and methotrexate in 4 patients and Mycophenolate mofetil in one patient. Grade I-II Acute GVHD occurred in all patients and was managed as per standard protocols.
Conclusions: AA is a life threatening disease and HSCT should be instituted at the earliest in all young adults whenever HLA matched donor is available. The challenges faced by HSCT unit are enormous, more so in a Government Hospital. This is an attempt to highlight the remarkable feat achieved in a short span of time in a resource poor setting in spite of the challenges.

Minimal Residual Disease in Multiple Myeloma

Pronamee Borah, Sangeeta Pathak, Nitin Dayal, Rahul Naithani

Introduction: Depth of remission is an important milestone in journey of patients with multiple myeloma. There is paucity of data on minimal residual disease (MRD) from India.
Aims & Objectives: To assess MRD status at different time points in patients with multiple myeloma.
Materials & Methods: Retrospective observational study. MRD analysis was performed by 10 color flowcytometry. MRD level of < 0.001 was considered negative.
Result: Total 40 MRD samples at various time points in 37 patients were analysed. Fourteen patients got MRD done from Day 60–150 (Majority on Day 100) of autologous HSCT. Eight patients were MRD negative while 6 patients had positive MRD (0.027 to 0.623). Four of these 6 patients progressed at a median of 2 years from ASCT while on maintenance therapy while 2/8 of MRD negative patient progressed. In 26 non transplant patients MRD was assesses (majority at end of 4 cycles of induction). Fourteen of these 26 patients had MRD negative while rest 12 had positive MRD (0.1 to 16.4). Three of these 12 patients proceeded to ASCT and became MRD negative at Day 100. Four (of 12) patients had relapse of myeloma. Follow-up data was missing for rest of the patients.
Conclusions: Flowcytometry based MRD analysis in multiple myeloma is feasible. Our small data points to poorer outcomes in patients with positive MRD and possible need for intensifying therapies. Autologous HSCT is useful modality to induce deeper remission in patients with multiple myeloma.

A Study on Outcome of Stool Culture and Sensitivity Surveillance in Patients Undergoing Hematopoietic Stem Cell Transplant

Shipla Roy, Shuvraneel Baul, Tuphan Kanti Dolai, Shubham Bhattacharya, Prakas Kumar Mandal, Sandeep Saha, Rajib De, Apurba, Abhishek, Kaustav, Chirasree

Introduction: Infections are the major cause of death in patients undergoing hematopoietic stem cell transplants (HSCT).These patients are at high risk for acquiring health care-associated infections. The use of empiric antibiotics during febrile neutropenia leads to a higher prevalence of multidrug-resistant organisms (MDROs) in this population. Surveillance strategies to detect colonization have been considered important tools for preventing and controlling the spread of MDROs in the hospital setting. Stool surveillance guided antibiotic usage leads to earlier defervescence in higher number of patients, while also reducing the need for second line antibiotics in bone marrow transplant patients. Our centre has a policy of performing surveillance stool cultures for all patients prior to transplant. This retrospective analysis is aimed at studying the surveillance of stool culture and sensitivity pattern on pre HSCT workup of patients.
Aims & Objectives: To assess the outcome of stool culture and sensitivity surveillance in patients undergoing hematopoietic stem cell transplant.
Materials & Methods: 19 patients were studied from January 2021 to September 2022 in the bone marrow transplant unit of NRS Medical College and Hospital. Stool culture and sensitivity of all the patients were sent on pre HSCT workup and the outcome was studied. Based on the culture and sensitivity report empirical antibiotic therapy was given during febrile neutropenia period after stem cell infusion.
Result: Median age of study population was 39 years, out of which 15 were male and four female. 18 underwent autologous HSCT and one allogenic. E Coli was the predominant organism found in 13 patients, E faecium and klebsiella pneumoniae were found each in one patient and no growth in four patients. Sensitive antibiotics were Amoxicillin + clavulanic acid, Piperacillin + tazobactam, Meropenem, Tigecycline and resistant antibiotics were Cefuroxime, Cefepime, Moxifloxacin and Trimethoprim + sulfamethoxazole. All the patients underwent successful HSCT.
Conclusions: Stool surveillance is important for preventing the spread of MDRO in HSCT patients and aids in guiding empirical

antibiotic therapy during febrile neutropenic period in post stem cell infusion phase.

Study of Pulmonary Function Tests in Newly Diagnosed Multiple Myeloma (NDMM) Patients Receiving Autologous Stem Cell Transplant (ASCT)

Alok Ranjan Pradhan, Manmohan Biswal, PC Dash, RK Jena

Introduction: Multiple myeloma accounts for 1% of all causes and about 10% of all hematological malignancies. Pulmonary complications which occurs in 30% to 60% of HSCT recipients constitute a major cause of post HSCT morbidity and mortality. Non infectious pulmonary complications have emerged as a major cause of post HSCT morbidity and mortality.
Performing PFT post HSCT, not only allows the early identification of Non-Infectious Pulmonary Complications (NIPCs), but also enables early preventive and therapeutic interventions in at risk patients.

This study conducted to find out the association of abnormal PFT towards the development of post HSCT NIPCs.
Aims & Objectives: To study the PFT in newly diagnosed patients receiving ASCT (both pre and post).
Its impact on pulmonary complications, morbidity and mortality.
Materials & Methods: Thirty-two cases of NDMM achieving satisfactory response (> VGPR) after induction therapy (VRD/VTD) received conditioning regimen of high dose Melphalan(200 mg/m2) followed by ASCT upfront. PFT, CXR, HRCT thorax were done prior to ASCT and post ASCT at 1st, 3rd, 6th, 12th month.
The pulmonary complications and mortality were recorded in all cases and compared and correlated.
Result: Pre-transplant mean values of FEV1, FVC, FEV1/FVC, TLC, DLCO were 94.5%, 81.5, 92, 4.29, 81 respectively. Out of 32 cases, 12 cases were having abnormal spirometry pattern (RESTRICTIVE PATTERN) and rest were without having any abnormality.
There was a consistent reduction of FEV1, FVC and DLCO upto 3rd follow up and all parameters had decreased at 3rd follow up.

At 12th month after ASCT, all parameters recovered to pre-transplant values except DLCO, which was in trend of recovery to pre-transplant values.

Total 6 patients died due to Non-Infectious Pulmonary Complications.
Conclusions: Pulmonary complications like NIPCs are the major causes of morbidity (10 out of 32,31%) and mortality (6 out of 32,18%).
Pre ASCT abnormal PFT (12 from 32, 37%) are positively correlated with above related morbidity (8 NIPCs from 12) and mortality (6 out of 12).

Transfusion Medicine

Role of Demographic Factors on Seroprevalence of Transfusion Transmitted Infections Among Blood Donors in a Tertiary Care Hospital—A Four Years Retrospective Study

Pawan Singh, Manju Daiya, Ashok Sangwaiya, Puja, Neerav Saini, Shailesh Kumar Mishra

Introduction: Blood donors belong to a heterogeneous group of people in society, differing in their demographic characteristics and the psychological factors that motivate their behavior.
It is important to analyze the various blood donor characteristics in order to manage blood donor programmes.
Aims & Objectives: The present study was conducted to determine the role of various demographic factors like age, sex and occupation on the seroprevalence of transfusion transmitted infections (TTIs) among the blood donors at a tertiary care hospital.
Materials & Methods: A 4 year retrospective study was conducted; all data were collected from blood bank records and included records of 1347 voluntary and 7451 replacement blood donors.
Screening of blood units was done by enzyme-linked immune sorbent assay (ELISA) method for Human Immune Deficiency Virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV). Syphilis was tested by rapid plasma resin (RPR) card test. Malaria was tested by antigen rapid diagnostic test.

Any sample found reactive was retested for confirmation.
Result: Total 8798 blood donor's samples were analyzed. 4.27% were females and 95.73% were males.
128 blood donors were found positive; prevalence of TTIs was at 1.45%. The overall positivity rates of anti-HIV, HBsAg, anti-HCV, anti-TP and MP were 0.19%, 0.80%, 0.40%, 0.06% and 0.01% respectively.

In the present study, overall seropositivity in replacement donors is less than voluntary blood donors.

The prevalence of TTIs was 1.47% for male and 1.06% for female in the donation population; the prevalence of TTI positive donations was highest in age group of 51–60 years. Regarding occupation, farmers showed the highest incidence (3.36%) of TTIs while businessmen (1.16%) ranked as the bottom.
Conclusions: Overall prevalence of TTI is more in voluntary blood donors as compared to replacement donors. So strict selection of donors and proper testing of donor's blood by using standard method is highly recommended to ensure safety for recipient. Other factors such as public awareness, vigilance of errors, educational and motivational programs is sure to help in decreasing the infections.

Overview of Voluntary Blood Donation Camps: An Experience in a Charitable Hospital

Chalana

Introduction: Voluntary blood donation camps are happy veins for blood banks worldwide. A blood bank faces many challenges in making blood donation safe, pleasant and memorable experience for the donor.
Aims & Objectives: Synopsis of blood donation camps across a year.
Materials & Methods: Retrospective study of blood donation camps was done for the period; December 2020 to December 2021 by retrieval of blood bank records.
Result: Maximum number of donors were in 2nd decade of life.

- Adverse reactions in donors were mild to moderate; mostly vasovagal
- Blood collection: Projected mean: 78.5, Actual mean: 61.2–- average gap being narrow.
- Predominant blood group among donors was O positive.
- Discard of bags [blood and its components] were mainly due to expiry followed by syphilis.

Conclusions: The closer the bed to the potential donor, the stronger is the likelihood of success of blood donation, possible only through Voluntary Blood Donation Camps.
Organizing blood donation camps is a perfect way to cater to the demand of blood.

Insights gained by analysis of conducted camps definitely showed a pathway for better future camps– in adopting better strategies to improve the overall quality and donation experience.

RESULTS:

Blood Donors ;total:2100

Age group	Male	Female	Total
16-32	359	02	361
23-27	530	04	534
28-32	439	03	442
33-37	421	02	423
38-42	280	03	283
43-47	29	04	33
48-52	16	02	18
53-57	04	01	05

A Rare Case of Hemolytic Disease of Fetus and Newborn Due To Maternally Derived Anti-E Alloantibody

Ayesha Sinha, Debapriya Basu, Mahua Reddy, Suvro Sankha Datta,Sabita Basu

Introduction: Hemolytic Disease of Fetus and Newborn (HDFN) may be caused by ABO isoagglutinin as well as alloantibodies directed against Rhesus and other minor blood group antigens. Clinically, it can present as mild anemia, hyperbilirubinemia, or manifest as severe anemia with hydrops and death of the fetus. The most commonly detected antibody in HDFN is anti-D whereas HDFN involving other blood group systems are often under-reported. Herein, we are reporting one such rare case of HDFN due to anti-E alloantibody.
Aims & Objectives: To highlight the importance of antibody screening in antenatal mothers irrespective of their RhD status.
Materials & Methods: A 3 days old term neonate of 2.8 kg presented with icterus and anemia. Sepsis screening and G6PD levels were normal. Blood samples of the baby and parents were sent to the immunohematology laboratory for evaluation. Blood grouping, direct and indirect antiglobulin tests (DAT/IAT) were performed by column agglutination technique. Extended RBC phenotyping was done by tube method using monoclonal rare antisera (anti C, c, E, e). Subsequently acid-elution was performed on the baby's red cells. Antibody titration was done using double dilution technique at anti-human globulin phase. All tests were performed according to the methods described in the AABB Technical Manual.
Result: Both mother and the baby were typed as B, RhD positive and father was typed as O, RhD positive. DAT of the baby was positive for IgG (IgG1 subtype with a titer of 100). Antibody identification test of the mother's plasma was positive, showing anti-E specificity with a titre of 1024 [Fig. 1]. Also, eluates from baby's red cells showed specificity to E antigen. Extended phenotyping showed that the baby and father shared similar phenotype of c and E antigens, which was absent in mother. The baby was diagnosed as a case of anti-E mediated HDFN and managed with double surface phototherapy. Two units of E-antigen negative PRBC (15 mL/Kg) were transfused and the baby was discharged on day12.
Conclusions: Antibody screening should be done in all antenatal mothers because HDFN can occur even if the mother and baby are typed identically for ABO/RhD. Unavailability of immunoglobulin in non RhD HDFN makes the situation more critical.

De Novo Mutation of the Beta Globin Gene IVS1-5(G > C) Position in Three Cases from West Bengal

Jyoti Shaw, Sunistha Bhattacharjee

Introduction: Beta thalassemia is the commonest single gene disorder prevalent in high percentage in eastern India. Inherited as autosomal recessive disease. However, few cases of sporadic inheritance are reported in beta thalassemia. In 1997, Waye et al. reported de novo mutation of the beta globin gene initiation codon ATG > AAG in Northern European boy. In 2018, Hasan et al. reported two cases of de novo mutation during prenatal screening test for thalassemia in two separate Bangladeshi family. One affected fetus had homozygous mutation at codon 26 and another fetus had homozygous mutation at IVS1-5 (G > C). In both of these cases father was normal, but mother was carrier for the mutation.
Aims & Objectives: Genetic study of three probands of beta thalassaemia trait by HPLC but behaving as thalassaemia intermedia.
Materials & Methods: Peripheral blood was collected from all the individuals after obtaining clinical history and informed written consent. Complete blood count was done in Sysmex XP-100 and Hemoglobin analysis was performed in BioRad Beta thal short program Variant –II. Then common mutations for alpha and beta globin genes were investigated in the DNA sample. Parenteral screening for beta mutation was also studied.
Result: Three families underwent mutation study. The hematological parameters were tabulated below. All the three probands were found to possess IVS1-5(G > C) at homozygous state. However parenteral study of HPLC showed that father is β-trait and mother is normal. We did not go for further study of HLA and STR based parentages testing because motherhood is beyond any doubt. Mother of all three probands has completely normal hematological parameters. All the three Fathers'are heterozygous for IVS1-5(G > C) mutation but mothers' carry the normal/wild type allele 'G'. Two probands and respective mother had high HbF and were found to be positive for HPFH mutation. One proband was TDT, another was NTDT while the third one never required transfusion till reported.
Conclusions: It is highly probable that DNA change occurred in the germline cells of the mother. This is first report of de novo of beta

mutation where mother is non- carrier. Such reports indicates that globin genes are one of the hotspots for DNA alterations in human genome.

Role of Alpha Mutation in Modifying the Phenotypes of Beta Thalassemia Trait

Sunistha Bhattacharjee, Jyoti Shaw, Anjumana Khatun, Maitreyee Bhattacharyya

Introduction: Thalassemia is the most common autosomal recessive genetic disease worldwide comprises of defective synthesis of globin chains. Individuals having single mutation are usually asymptomatic and are denoted as beta thalassemia trait (

Aims & Objectives: To investigate the beta and alpha mutation in a group of beta thalassemia carriers behaving as intermedia.

Materials & Methods: This study was conducted from the period of early 2017 to mid-2022 involving 40 thalassemia cases behaving as intermedia. A detailed history and thorough clinical examination was done for presence of anaemia, jaundice and splenomegaly. 2 ml blood was taken after written informed consent from the patients. Complete Hemogram (SYSMEX XP-100) and HPLC (BIORAD Variant II beta-thalassemia short program) was performed.

To rule out associated other haemolytic conditions giving rise to intermedia phenotype G6PD estimation, incubated osmotic fragility test and direct coomb's test was done in all the cases. The patients negative for all the above tests were included in this study for further analysis. ARMS PCR was done for common beta mutations and Gap-PCR (Lie et al.) for alpha triplication. Further Sanger sequencing was performed where ARMS PCR failed to answer for the beta status.

Result: Out of 40 patients 25 were transfusion independent and 15 required occasional blood transfusion. Only one patient aged 14 years needs regular blood transfusion. Most of the patients had jaundice and splenomegaly.

Genetically, all patients were carriers of a single alteration in the HBB gene corresponding to 4 different mutations—IVS-1–5(G > C), CD 15 (G > A), CD 30, IVS-1–130 (G > C). Out of 40 patients 29 were found to have Alpha globin gene triplication (ααα anti-3.7). HPLC study shows the mean Hb, HbF and HbA2 value in these cases were 7.72, 4.56 and 5.23 respectively whereas individuals without triplication have lower HbF (3.39) and other parameters were at par.

Role of Tpe in Wilson's Disease Presenting with Fulminant Liver Failure: Case Report

Apalak Garg, Divjot Lamba, Rekha Hans, Sadhna Lal, Rati Ram Sharma

Introduction: Wilson's disease is an autosomal recessive inherited disorder of copper metabolism. Therapeutic plasma exchange (TPE) provides rapid chelation of copper in Wilson's disease (WD) preventing further aggravation of liver failure. TPE in fulminant WD is mentioned as a category 1 indication in the American Society for Apheresis (ASFA) registry.

Aims & Objectives: To study the role of TPE as a bridge to liver transplant and improvement in clinical condition of the patient following the procedure.

Materials & Methods: We report two cases of acute WD that rapidly progressed to life threatening multi organ failure and role of TPE in acute management of WD to act as a bridge therapy for liver transplantation.

Result: Case 1-Patient 6 year male child weight 23 kg reported to us in July 2022 with S. Bilirubin (total)—35.1 mg/dl and conjugated bilirubin 26.2 mg/dl, PT was 22%, PTI was 28%, APTT 59.3 s (all were prolonged). Patient's 24 h urine copper levels were 1455ug/24 h (< 60ug >

Case 2- Patient aged 11 year old male child with weight of 35 kg and S.Bilirubin (total)—41.6 mg/dl and conjugated bilirubin 27 mg/dl, PT was 35%, PTI was 39%, INR 2.50, APTT 86.7 s. Patient's 24 h urine copper levels were 1320ug/24 h (< 60ug >

Conclusions: Multiple modalities to reduce copper load to prevent fulminant liver failure in Wilson's disease are tried i.e. TPE along with D- penicilamine and to serve as a bridge therapy for liver transplantation. Our case highlights the late referral of such patients to institutes with facilities for liver transplantation when fulminant disease progresses to multi organ faliure and death in few days of time.

INVESTIGATIONS	PATIENT NAME-DIVYANSHU (total 10 procedures)		PATIENT NAME-SAHIL (total 11 procedures)	
	Pre-Cycle values	Post- Cycle completion values	Pre-Cycle values	Post- Cycle completion values
PT (12–15 s)	60.7	25.4	35	25.1
PTI (80–100%)	25.4	46	25.1	49
APTT (27–33 s)	44	38.6	86.7	35.1
INR (0.82–1.1)	3.43	1.88	2.5	2.11
Total S. Bilirubin (0.1–1.2 mg/dl)	36.16	7.51	41.46	12.03
Cong. Bilirubin (0-0.3 mg/dl)	21.18	4.45	26.98	5.52
S. Albumin (3.5-5.3 g/dl)	2.57	2.72	2.5	3.3
S. Protein (6.4–8.3 gm %)	4.5	4.6	4.6	5.1
SGOT (AST) 2–40 IU/L	98	81	117	90
SGPT (ALT) 2-40 IU/L	26	28	43	54
24 h Urine Copper (< 60 µg/24 h)	1455		1320	
S. Cerruloplasmin (22–58 mg/dl)	15.2		13.8	

Prevalence and Predictors of Platelet Refractoriness in Haematology Inward Patients

Aryabhatta Sadhu, Mukul Aggarwal, Hem Chandra Pandey, Manoranjan Mahapatra, Poonam Coshic

Introduction: Haematological patients often require regular, repeated platelet transfusions. Platelet transfusion failure to increase platelet count can be due to immune and nonimmune factors and is associated with poor outcomes. The incidence, determinants, and prognostic variables of platelet refractoriness are unknown in Indian patients.

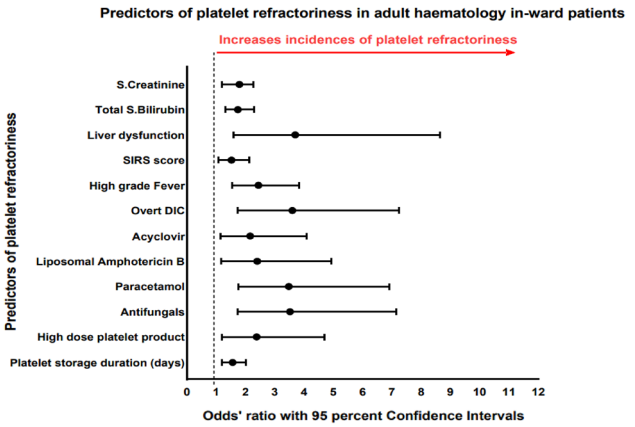
Aims & Objectives: To determine the prevalence and predictors of platelet transfusion refractoriness at a tertiary care center.

Materials & Methods: A prospective observational study in adult haematology patients who received platelet transfusion on two

separate occasions, 24-h apart were analysed for response to platelet transfusions during their in-hospital stay (median 28 days) using corrected count increment (CCI)-24 h, 2 consecutive 24 h CCI < 5000 classified as Refractory, in such cases, 2 further 1 h CCI were calculated; if CCI < 7500, classified as immune/mixed refractory, rest as non-immune refractory. Clinical scales like Overt DIC (ISTH score > 5), SIRS (sofa) (score ≥ 2), degree of fever and splenomegaly were used to quantify the severity of observations. Statistical analysis done using STATA 12.

Result: Study cohort (n = 168 patients) received 3138 RDP equivalence units. The prevalence of platelet refractoriness was (55.95%) with (61.0%) non-immune and (39.0%) immune refractoriness. Predictive factors for the development of refractoriness were usage of antifungals (OR 3.52, CI 1.74–7.14, p = < 0.0001), Paracetamol (OR 3.48, CI 1.75–6.92, p = < 0.0001), Liposomal Amphotericin-B (OR 2.4, CI 1.17–4.93, p = 0.017), Acyclovir (OR 2.16, CI 1.15–4.08, p = 0.018); Platelet characteristics: storage duration (OR 1.56, CI 1.2–2.0, p = 0.001) and higher dose product (OR 2.38, CI 1.2–4.7, p = 0.013); clinical factors: overt DIC (OR 3.6, CI 1.74–7.24, p = 0.001), high grade fever (OR 2.44, CI 1.54–3.84, p = < 0.0001), SIRS score (OR 1.52, CI 1.08–2.13, p = 0.018), liver dysfunction (OR 3.7, CI 1.58–8.64, p = 0.003), total serum bilirubin (OR 1.74, CI 1.3–2.3, p = < 0.0001), and creatinine (OR 1.79, CI 1.2- 2.27, p = 0.005).

Conclusions: Refractoriness affected 56% of hematology patients, with 61% due to non-immune causes. Platelet product quality, medications, overt DIC, high SIRS (SOFA) score, and raised total serum bilirubin were predictors for the development of refractoriness. Hence, platelet refractoriness should be anticipated with these risk factors and their resolution might contribute to a reduction in the burden of the problem.



Granulocyte transfusion in children with severe neutropenic sepsis: observations from a tertiary care centre in Western India

Sneha Shinde, Swathi Krishna, Purvaja Kubde, Dhara Shah, Vaibhav Chadhha, Trupti Dhabale, Ritika Khurana, Purva Kanvinde, Minnie Bodhanwala, Sangeeta Mudaliar

Introduction: Bacterial and fungal infections are a major cause of mortality in neutropenic children, especially in developing countries. Granulocyte transfusions have been a topic of debate since its inception. It is well known that due precautions should be taken for concurrent administration of granulocytes with Amphotericin B and in patients with acute lung pathology. In this single-centre retrospective observational study, we present our data on children receiving granulocyte transfusion for severe neutropenic sepsis.

Aims & Objectives: To determine the effectiveness and safety of granulocyte transfusions in cancer patients with severe neutropenic sepsis.

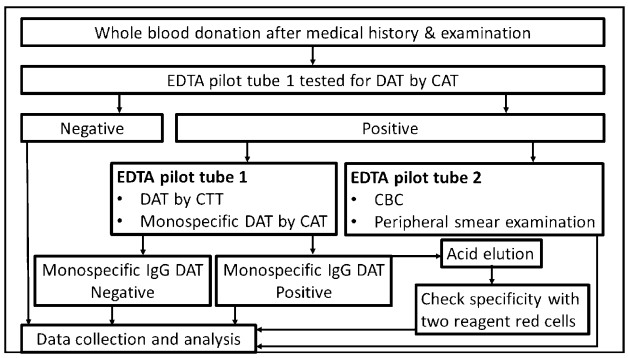
Materials & Methods: Patients having an absolute neutrophil count of less than 0.05 × 10^3/microL with life threatening sepsis in the form persistent high grade fever or hemodynamic instability despite using highest anti microbials, were considered for granulocyte transfusion at a dose of 10 ml/kg. Granulocytes were obtained by apharesis either from centrifugation (buffy coat) or by mobilisation from voluntary donors with G-CSF and dexamethasone. A chest Xray was done prior to transfusion to look for any underlying acute lung pathology.

Result: Our study included a total of 21 patients, with a male to female ratio of 2:1. Majority of the patients were in the age group of 2–8 years. Acute lymphoblastic leukemia was found to be the most common (45.4%) followed by Burkitt's lymphoma (19%), Acute myeloid leukemia (14%), DLBCL (3.7%) and one patient each of Hepatoblastoma and Osteosarcoma.

3 out 21 patients received granulocytes obtained by mobilisation from a single donor whereas the rest from buffy coat.

Favourable response was seen in 18 out of 21 (85.7%) children with resolution of sepsis within 3–4 days. 3 children succumbed to sepsis. 1 patient with an underlying lung infection who was already receiving Amphotericin B developed increased respiratory support requirements post granulocyte transfusion.

Conclusions: Granulocyte transfusions are well tolerated and can help neutropenic patients tide over the acute crisis and reduce mortality due to sepsis. Granulocytes obtained through buffy coat were also found to be effective and can serve as a rescue measure in resource limited settings.



A Cross-Sectional Survey on Clinical Practices in the Management of Autoimmune Hemolytic Anemia-First Report From India

Suvro Sankha Datta; Special interest group of immunohaematology

Introduction: Autoimmune hemolytic anemia (AIHA) is a decompensated acquired hemolysis caused by the host's immune system acting against its own red cell antigens. However, there are no standard diagnostic criteria for AIHA and its subtypes until recently few recommendations have been obtained from an international consensus group. Furthermore, being a relatively rare condition, the evidence for clinical practice is limited in India. This is the first of its kind national survey across India where clinical responses have been collected from the clinical hematologists across the country.

Aims & Objectives: The main aim of the survey was to capture the real-world scenarios of the clinical practice of AIHA in India and address the deficiencies that are caused because of non-adherence to the standard international guidelines.
Materials & Methods: In this cross-sectional study, a structured, 26-question online survey was conducted amongst clinical hematologists in India. The survey was administered in English using a Google Form which was distributed to clinical hematologists through e-mail between January to March, 2022. The respondents were not anonymous; therefore, all participants reviewed the informed consent page and provided their consent. Most of the questions required only a single response while few had an option of more than one response. Not all questions were responded to due to skip logic used in the software, thus the denominator changed for some responses. Descriptive statistical analysis was performed.
Result: The survey response rate was 48.2% (53/110), 69.8% (37/53) have diagnosed and managed more than ten AIHA cases in the last 3 years with a female preponderance. There was considerable variability in response. While 56.6% (30/53) of respondents do have the access to the facilities to subtype AIHA cases; 32.1% (17/53) of them would prefer administering high dose steroids for 6 weeks or more in nonresponding patients, and only 45.3% (24/53) would assess the risks of thrombosis in AIHA. There is unanimous agreement among the participants that health-related quality of life should be taken into consideration in patients and the need for a national registry of patients with AIHA in India. The survey highlighted inconsistencies is showed in the attached Figure.
Conclusions: The current national survey showed that some aspects of AIHA management were consistent but there were significant variations observed in certain clinical practices. A effort is needed to establish a national patient registry which could potentially standardize AIHA management in India.

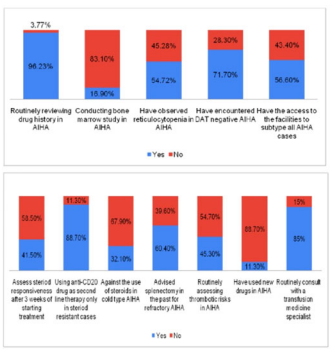


Table.1 Variation between international guidelines and survey exhibited clinical practice

	Guideline recommendations	Survey findings
1.	Should subtype all diagnosed AIHA cases as warm/cold/mixed types before starting the treatment.	Only 56.6% (30/53) physicians have the access to the facilities to subtype AIHA cases in India.
2.	Steroid responsiveness should be assessed after 3 weeks of starting treatment.	Merely 41.5% (22/53) respondents would prefer to assess the steroid responsiveness after 3 weeks. 32.1% (17/53) would prefer to continue the steroid at the dose of 1mg/kg/day beyond three weeks, while 26.4% (14/53) of respondents would not wait till three weeks for the response assessment.
3.	Steroids are not effective in CAD as first-line therapy	Only 32.1% (17/53) respondents indicated that they would never use steroids in CAD as first-line therapy.
4.	Thrombotic risks should be assessed in AIHA patients especially in elderly.	Very few respondents (24/53; 45.3%) would assess the risks of thrombosis in elderly AIHA patients and consider VTE prophylaxis.
5.	Use of novel agents might be considered in relapse, refractory AIHA cases.	As low as 11.3% (6/53) haematologists had an experience of using novel agents in AIHA. Most of the respondents (88.7%) remain doubtful regarding the use of such therapy in India because of the high cost of these drugs.

GCSF Induced Thrombocytopenia: A Rare Find

Juhi Mehrotra, Santanu Sen

Introduction: Peripheral Blood Stem Cell collection is widely used for stem cell harvesting and is generally considered a very safe option. At times mild decrease in platelet counts has been reported, mainly attributed to leukapheresis process itself.
Aims & Objectives: We report a case of significant thrombocytopenia seen in a patient during stem cell mobilization.
Materials & Methods: 3 year old scheduled for stem cell harvest was started on GCSF at 10mcg/kg daily with pre-procedure count of 2.14 lakhs and negative for any viral serology.
Result: Day 3 of G-CSF platelet count was 10,000. There was no bleeding and clinical examination was unremarkable. Infective causes were ruled out. Since the thrombocytopenia was unexplained, we repeated a CBC after 12 h and platelet count improved to 26,000. 4th day GCSF, the platelets dropped to 11,000. Since patient needed central lines for the harvest, platelets were transfused which reflected as a 42,000 in the next CBC. As we found no cause for the thrombocytopenia, we reviewed the literature and surmised that this could be the direct effect of GCSF on the platelet count. Day 5 CBC showed platelet counts of 22,000 and stem cells were successfully collected. Though the platelet counts post stem collection did show a downward trend of 34,000 at 24 h., 21,000 at 48 h, and 14,000 at 72 h. after the last dose of G-CSF, on the subsequent day, platelets showed spontaneous increase to 64,000. Subsequent CBCs were normal with complete recovery of platelet counts.
Conclusions: PBSCs mobilized by G-CSF is widely used.GCSF induced thrombocytopenia is not well known. Cases have been reported infrequently, where GCSF use has led to fall in platelet counts which recovered spontaneously on stopping the drug. It is also relevant that GCSF is often used post-transplant to reduce the period of neutropenia and prevent infections, thus it is important to recognise that delay in platelet recovery might be a direct effect of GCSF itself.

Comparative Analysis of Haematological Parameters of First-Time and Repeat Blood Donors

Mayank Kumar, Ranvijay Singh, Dinesh Kumar Singh

Introduction: Blood transfusion services form an essential component of any healthcare system and it is imperative to provide adequate and safe blood for management of patients. Voluntary blood donors form the backbone of this service. However, regular donation by such voluntary donors may cause significant depletion of iron stores in the body. This has the potential to adversely affect the donor's health, and also to lower the quality of blood being collected subsequently. The prompt detection of subclinical iron deficiency in voluntary blood donors is the need of the hour.
Aims & Objectives: To compare and analyse the difference in haematological parameters of first-time and repeat blood donors.
Materials & Methods: A descriptive study was conducted by the Department of Pathology and Blood Bank at ASMC, Ayodhya. After prospective donors were assessed for suitability of blood donation, written informed consent was obtained, and 5 ml venous blood was collected into an EDTA-anticoagulated vial via the antecubital fossa. Complete blood count was performed within one hour of collection using an automated haematology analyser.
The generated data was compiled in MS Excel software. Statistical analysis was performed to determine the significant differences, if any.
Result: The study included 250 participants, out of which 100 were repeat blood donors. Statistical analysis showed significant differences for mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) between groups defined by number of donations. The difference was most significant between the donors having 5 or more donations compared to donors having no previous donations. No significant difference was observed for other parameters.
Conclusions: Haemoglobin level, which is routinely used to screen prospective blood donors, is considered a poor marker for identifying iron deficiency as lowering of haemoglobin occurs at a very late stage of iron depletion. Donors who have previously donated blood multiple times have significantly lower MCV and MCH. These donors, with haemoglobin values within normal range, are most susceptible to having subclinical iron deficiency, which needs to be identified and managed pre-emptively, before development of iron deficiency anaemia. This is necessary in order to retain regular and repeat voluntary blood donors, and also to ensure adequate quality of collected blood.

CCN3 as a Circulatory Diagnostic/Prognostic Biomarker in Osteosarcoma Patients: A Follow-Up Study

Manish Yadav, Archana Raikwar

Introduction: Osteosarcoma is the world's fifth most common malignant disease in adolescence. Despite of novel and innovative therapies and tremendous research till date, the early diagnosis/prognosis of the osteosarcoma is lacking, leads to higher mortality, morbidity and ultimately the socioeconomic loss. Focusing the emerging area of translational oncology, this molecular study aimed to evaluate the CCN3 proteins level as a diagnosis/prognosis marker in primary osteosarcoma patients.
Aims & Objectives: To evaluate the Gap and Cast indices as a predictor of efficacy of plaster cast in management of displaced diaphyseal fractures of forearm and legTo calculate Cast and Gap index in patients managed by plaster casts for displaced diaphyseal fractures of both bone forearm and leg.
2) To evaluate the change in Cast and Gap indices (if any) during follow up in adults and pediatric patients managed by plaster cast for displaced diaphyseal fractures of both bone forearm and leg.
3) To analyse the threshold value of Cast and Gap indices as a predictor of loss of reduction of displaced diaphyseal fractures of both bone forearm and leg.
Materials & Methods: A total of 40 cases of primary osteosarcoma and 40 controls were enrolled according to inclusion/exclusion criteria. All the patients underwent surgical procedures followed by pre and post-chemotherapy as per institutional standard protocols. The serum CCN levels were measured at different initial follow-ups in cases (once in controls) and were correlated with the clinic-radiological profile of the patients.
Result: All the demographics data between cases and controls found statistically insignificant differences. The mean serum CCN3 level showed a statistically elevated level in osteosarcoma cases in comparison to controls, which were decreases subsequently during follow-ups while treatment in most of the cases. By performing ROC analysis between the baseline samples of cases with controls, statistical significant differences were observed. Furthermore, statistical significant correlations were found between different grades of osteosarcoma patients with their serum CCN3 levels at various follow-ups during the treatment.
Conclusions: The elevated level of CCN3 protein in serum may be used as a promising and non-invasive diagnostic/prognostic molecular biomarker in primary osteosarcoma.

Profile Of Fetal Outcome in Rh-Positive Mothers With Indirect Coomb's Test Positive: A Report Of 3 Cases

Vageeshagarg, Saqib Ahmed, Aparna Bhardwaj, Brijesh Thakur, Seema Acharya

Introduction: In majority of cases, routine antenatal antibody screening is done only for Rh(D) negative mothers while Rh D positive mothers are often overlooked which may lead to a serious delay in diagnosing Hemolytic Disease of Fetus and Newborn (HDFN) due to the rarity of antibodies like anti-c, C, e, E, or Kell, Kidd, Duffy, MNS, Lutheran, Diego, Xg.
Aims & Objectives: This study was conducted with an aim of studying the fetal outcome amongst RhD positive mothers having irregular antibodies.
Materials & Methods: A total of 200 antenatal Rh positive patients were screened over a period of 10 months and were subjected for Indirect Coomb's test (ICT). Patients who were ICT positive were further evaluated for ICT titres and the type of irregular antibodies by employing 11 cell antibody cell panel. These patients were then followed throughout the pregnancy to assess the fetal outcome.
Result: Of 200 Rh positive patients, 3 patients were ICT positive and were followed regularly during their Antenatal visits. One of the patient who was Gravida 5 with previous history of transfusion and ICT titre of 1:128, had Anti-Kell antibody, delivered preterm with massive fetal ascites and jaundice. The baby was Direct Coomb's Test (DCT) positive and underwent repeated exchange transfusion but ultimately baby succumbed. The other two patients who were ICT positive had a titre of 1:8 (Gravida 2) and 1:4 (Gravida 3) respectively. On screening, these patients had Anti-M and Anti-Kidd(Jka) antibodies respectively, underwent term delivery. Both the babies were healthy, but the baby of patient with Anti-M antibody had mild anemia and prolonged jaundice.
Conclusions: Patients with Anti- Kell antibody have poor fetal outcome compared to those who have anti-Kidd and anti-M antibodies. Thus, screening of maternal serum antibodies in Rh positive mothers must be formulated as a protocol to prevent perinatal morbidity and mortality thus improving the fetal outcome.

Flow Cytometric Analysis of Platelet-Leukocyte Aggregates And Hematological Parameters in Pre and Post Plateletpheresis Donors

Subarna Sharma Pinky, Anjali Sharma, Mukul Singh

Introduction: Single donor platelet apheresis (SDAP) has grown steadily due to its wide use in hematological malignancies and platelet-related diseases. SDAP procedure is usually well tolerated by donors and are preferred without any significant complications. However post procedure safety issues regarding the formation of platelet leukocyte aggregates and changes in hematological values in the donor's blood circulation are not well assessed. We aimed to analyze platelet-leukocyte aggregates as well as changes in hematological parameters in pre and post plateletpheresis donors by using different platelet surface antigens on flow cytometry.
Aims & Objectives: To analyze the expression of CD41, CD42a and CD61 on platelet-leukocyte aggregates in pre and post plateletpheresis donors by flow cytometry and to compare the variation in hematological parameters in them.
Materials & Methods: This observational study included 30 healthy single donor platelet apheresis donors who volunteered in the Department of Pathology and Transfusion Medicine, VMMC and Safdarjung Hospital, NEW DELHI. Donor selection was based on National Blood Transfusion Council Guidelines 2019. Pre and post donation blood samples were collected and analyzed for changes in hematological parameters and flow cytometric evaluation of platelet-leukocyte aggregates by using CD41, CD42a, CD61 antigens.
Result: The mean age group was 20–47 years and all the donors were male. Among thedifferent platelet-leukocyte aggregates observed via flow cytometry, CD42a-positive platelet-neutrophil aggregates and CD61-positive platelet-neutrophil aggregates PNA) werestatistically significant $p = 0.001$ and $p = 0.043$ respectively. The rest of the platelet-leucocytecomplexes were statistically insignificant. Among the hematological parameters, post-donation platelet count ($p = 0.020$) and absolute lymphocyte count ($p = 0.007$) have shownsignificant reduction. Changes in other hematological parameters were not significant statistically.
Conclusions: In our study, Single Donor Platelet Apheresis (SDAP) donors had a significantimmediate post-procedure reduction of platelet count and absolute lymphocyte count andsignificant increase in PNA formation. However, the evidence of platelet activation andformation of PNA may predispose certain donors to pre-thrombotic complications.Monitoring of donor's hematological values for a longer period of time and more prospective studies are recommended to establish donor safety guidelines.

High Throughput Genetic Screening of Beta Thalassemia Cohort in West Bengal

Jyoti Shaw, Sunistha Bhattacharjee, Anjumana Khatun, Maitreyee Bhattacharyya

Introduction: Beta-thalassemia is one of most common autosomal recessive genetic disorders. High prevalence is reported in populations in the Mediterranean, Middle East, Central Asia, Indian subcontinent, and Far East. The phenotypic severity of the disease depends on the particular type of genetic mutations that a patient harbors. Around 950 variants in beta globin gene are documented in human globin gene server, of which 200 mutations are commonly found worldwide. Five most common mutations like IVS1-5(G > C), CD 8/9, CD41/42, CD30, CD26 are said to define almost 90% of thalassemia population in India but 10–15% of cases remain undefined.

Aims & Objectives: To determine the beta globin gene mutations which cannot be determined by ARMS PCR method.

Materials & Methods: In a prospective study from 2015 to 2022, suspected thalassemia cases are investigated for alpha and /or beta globin gene mutation. Peripheral blood was collected after informed consent and ARMS PCR was done for assessing beta gene mutation and GAP-PCR was employed for analyzing the common alpha mutations. But in 15% of cases, mutation status could not be established. These cases were further subjected to DNA sequencing of beta globin gene following the manufacturer's protocols.

Result: DNA sequencing for beta globin gene was done in 120 samples. The results were given in the table below. Common mutation like IVS1-5 (G > C) was confirmed in 68 cases. Out of this 12 were confirmed for homozygous state and 56 were found heterozygous. These 56 cases are either beta carrier or compound heterozygous with other mutation. CD15 may be detected by ARMS PCR but in our lab it is usually detected by DNA sequencing. CD15 is found in three cases. In 77 cases, twelve uncommon or rare mutations were detected (Table). Few of these uncommon mutations are of β° type like IVS1-130, IVSII-850 and Hb Monroe and Two β + and seven Hb variant were also identified by DNA sequencing.

Conclusions: Sometimes even the mutation status of common mutation like IVS1-5 remains inconclusive by ARMS PCR technique and DNA sequencing is done for confirmation. Moreover, thorough DNA sequencing of beta globin gene enabled us to determine the mutation status of the suspected thalassemia cases which otherwise remained undiagnosed and help treat patients better.

Table : Results of DNA sequencing of Beta globin gene

Mutation	Type	Status	Number	Reason for sequencing
IVS1-5(G>C)	β^+ severe	Homo	12	ARMS PCR inconclusive
		Hetero	56	Normal/no result in ARMS PCR.
CD 15	β^0	Homo	1	Not listed in six common mutation by ARMS PCR.
		Hetero	2	
-90	β^+	Homo	3	Beyond the scope of ARMS PCR
		Hetero	4	
Beta nt -42	Co-occurrence with a β^0	Hetero	4	
Hb Monroe	β^0	Hetero	4	
Cap site +1(A>C)	β^{++}(silent)	Hetero	2	
IVS1-130	β^0	Homo	2	
		Hetero	4	
HbM Saskatoon	Hb variant	Hetero	1	
HbD Iran	Hb variant	Hetero	3	
Hb Randwick	Hb variant	Hetero	1	
Hb Stockholm	Hb variant	Hetero	1	
IVSII-666	polymorphism	Hetero	32	
IVSII-850	β^0	Hetero	13	
Hb Renert (CD 133)	Hb variant	Hetero	3	
			Total - 148	

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